

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
3 March 2005 (03.03.2005)

PCT

(10) International Publication Number
WO 2005/019454 A1

(51) International Patent Classification⁷: **C12N 15/12**,
C07K 14/705, 14/47, C12N 5/10, C12P 21/02, C12Q 1/02,
1/68, G01N 33/566, 33/53

(21) International Application Number:
PCT/CA2004/001550

(22) International Filing Date: 23 August 2004 (23.08.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/497,096 22 August 2003 (22.08.2003) US

(71) Applicant (for all designated States except US): **NEU-
ROMEDTECHLOGIES, INC.** [CA/CA]; 2389
Health Sciences Mall, Suite 301, Vancouver, British
Columbia V6T 1Z4 (CA).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **PARKER, David**
[CA/CA]; 25343 - 100th Avenue, Maple Ridge, British Co-
lumbia V2W 1S3 (CA). **XU, Xianghong** [CA/CA]; 3379
West 22nd Avenue, Vancouver, British Columbia V6S 1J1
(CA). **KHAWAJA, Afsheen** [CA/CA]; 7263-122A Street,
Surrey, British Columbia V3R 3N9 (CA). **SNUTCH, Ter-
rance, P.** [CA/CA]; 3963 West 24th Avenue, Vancouver,
British Columbia V6S 1M1 (CA).

(74) Agents: **ROBINSON, Christopher, J.** et al.; Fetherston-
haugh & Co., Box 11560 Vancouver Centre, Suite 2200,
650 West Georgia Street, Vancouver, British Columbia
V6B 4N8 (CA).

(81) Designated States (unless otherwise indicated, for every
kind of national protection available): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, 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, NA, NI, NO, NZ, OM, PG,
PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM,
TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM,
ZW.

(84) Designated States (unless otherwise indicated, for every
kind of regional protection available): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI,
SK, TR), OAPI (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 "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: NOVEL RAT CALCIUM CHANNEL SUBUNITS AND RELATED PROBES, CELL LINES AND METHODS

(57) Abstract: Novel sequences for calcium channel δ_2 8-2 and δ_2 8-3 subunits are provided. Also provided are cell lines that express the novel calcium channel subunits of the invention. These cells may be used for identifying compounds capable of stimulating or inhibiting the activation of the calcium channels.



WO 2005/019454 A1

NOVEL RAT CALCIUM CHANNEL SUBUNITS AND RELATED PROBES, CELL LINES AND METHODS

Cross-Reference to Related Application

[0001] This application claims benefit of U.S. provisional application 60/497,096 filed 22 August 2003. The contents of this document are incorporated herein by reference in their entirety.

Technical Field

[0002] The present invention relates to novel calcium channel $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 DNA and protein compositions, and to the expression of these compositions in cell lines for use in evaluating calcium channel function and in screening for agonists/antagonists for these channels.

Background Art

[0003] Voltage-gated calcium channels are a heterogeneous family of membrane proteins, which respond to depolarization by opening a calcium-selective pore through the plasma membrane. The influx of calcium into cells mediates a wide variety of cellular and physiological responses including excitation-contraction coupling, hormone secretion and gene expression. In neurons, calcium entry directly affects membrane potential and contributes to electrical properties such as excitability, repetitive firing patterns and pacemaker activity. Calcium entry further affects neuronal function by directly regulating calcium-dependent ion channels and modulating the activity of enzymes such as protein kinase C and calcium-dependent calmodulin-dependent protein kinase II. Furthermore, an increase in calcium concentration at the presynaptic nerve terminal triggers the release of neurotransmitters. Calcium entry also plays a role in neurite outgrowth and growth cone migration in developing neurons and is implicated in long-term changes in neuronal activity. In addition to the variety of normal physiological functions mediated by calcium channels, they are also implicated in a number of human disorders. Recently, mutations identified in human and mouse calcium channel genes were found to account for several disorders including, familial hemiplegic migraine, episodic ataxia type 2, cerebellar ataxia, absence epilepsy and seizures. (See, for example, Fletcher, C. F., *et al.*, *Cell* (1996) 87:607-617; Burgess, D. L., *et al.*, *Cell* (1997) 88:385-392; Ophoff, R. A., *et al.*, *Cell* (1996) 87:543-552; Zhuchenko, O., *et al.*, *Nature*

Genetics (1997) 15:62-69. The clinical treatment of some disorders has been aided by the development of therapeutic calcium channel modulators or blockers. Janis, R. J. and Trigg, D. J. (1991) in Calcium Channels: Their Properties, Functions, Regulation and Clinical Relevance, CRC Press, London).

[0004] Native calcium channels are classified by their electrophysiological and pharmacological properties as either high voltage-activated (L, N, P, and Q types) or low voltage-activated channels (T-type). R-type channels have biophysical properties similar to both high and low voltage-activated channels. (For reviews see McCleskey, E. W. and Schroeder, J. E., *Curr. Topics Membr.* (1991) 39:295-326, and Dunlap, K., *et al.*, *Trends Neurosci.* (1995) 18:89-98.) T-type channels are a broad class of molecules that transiently activate at negative potentials and are highly sensitive to changes in resting potential. The L, N, P and Q-type channels activate at more positive potentials and display diverse kinetics and voltage-dependent properties. There is some overlap in biophysical properties among the high voltage-activated channels, consequently pharmacological profiles are useful to further distinguish them. L-type channels are sensitive to dihydropyridine (DHP) blockers, N-type channels are blocked by the *Conus geographus* peptide toxin, ω -conotoxin GVIA, and P-type channels are blocked by the peptide ω -agatoxin IVA from the venom of the funnel web spider, *Agelenopsis aperta*. A fourth type of high voltage-activated Ca^{2+} channel (Q-type) has been described, although whether the Q- and P-type channels are distinct molecular entities is controversial. Conductance measurements of several types of calcium channels have not always fallen neatly into any of the above classes and there is variability of properties even within a class, suggesting that additional calcium channels subtypes remain to be classified.

[0005] Biochemical analyses show that neuronal calcium channels are heterooligomeric complexes consisting of three distinct subunits (α_1 , α_2 and β) (reviewed by De Waard, M., *et al.*, in Ion Channels, Volume 4, (1997) edited by Narahashi, T., Plenum Press, New York). The α_1 subunit is the major pore-forming subunit and contains the voltage sensor and binding sites for calcium channel blockers. The mainly extracellular α_2 is disulphide-linked to the transmembrane α_1 subunit and both are derived from the same gene and are proteolytically cleaved *in vivo*. The β subunit is a non-glycosylated, hydrophilic protein with a high affinity of binding to a cytoplasmic region of the α_1 subunit. A fourth subunit, γ , is unique to L-type calcium channels expressed in skeletal muscle T-tubules. The isolation and characterization of γ -subunit-encoding cDNA's is described in U.S. patent No. 5,386,025, which is incorporated herein by reference.

[0006] The DNA's encoding the amino acid sequences of seven different types of α_1 subunits (α_1A , α_1B , α_1C , α_1D , α_1E , α_1F and α_1S) and four types of β subunits (β_1 , β_2 , β_3 and β_4) have been cloned. (Reviewed in Stea, A., *et al.*, "Voltage-gated calcium channels" in Handbook of Receptors and Channels (1994) Edited by R. A. North, CRC Press). PCT Patent Publication WO 95/04144, which is incorporated herein by reference, discloses the sequence and expression of α_1E calcium channel subunits.

[0007] In some expression systems the α_1 subunits alone can form functional calcium channels although their electrophysiological and pharmacological properties can be differentially modulated by coexpression with any of the four β subunits. Until recently, the reported modulatory affects of β subunit coexpression were to mainly alter kinetic and voltage-dependent properties. It has now been shown that β subunits also play crucial roles in modulating channel activity by protein kinase A, protein kinase C and direct G-protein interaction. (Bourinet, E., *et al.*, *EMBO J.* (1994) 13:5032-5039; Stea, A., *et al.*, *Neuron* (1995) 15:929-940; Bourinet, E., *et al.*, *Proc. Natl. Acad. Sci. (USA)* (1996) 93:1486-1491.)

[0008] The $\alpha_2\delta$ subunits comprise at least four types encoded by different genes. The first subunit identified was $\alpha_2\delta$ -1 from rabbit skeletal muscle (Ellis, *et al.*, *Science* (1988) 241:1661-1664). Five tissue-specific splice variants exist (Angelotti, T. and Hofmann, F., *FEBS Lett.* (1996) 397:331-337). $\alpha_2\delta$ -2, -3 and -4 have been identified recently in human and mouse (Klugbauer, N., *et al.*, *J. Neuroscience* (1999) 19:684-691; Qin, N., *et al.*, *Mol. Pharmacol.* (2002) 62:485-496). These $\alpha_2\delta$ subunits share 30% to 56% amino acid identity with the $\alpha_2\delta$ -1 subunit as well as several structural motifs, such as similar hydrophobicity profiles, glycosylation sites and cysteine residues. $\alpha_2\delta$ -1 and $\alpha_2\delta$ -2 subunits are expressed in many tissues including the brain and heart, while $\alpha_2\delta$ -3 is found only in the brain (Klugbauer, *et al.*, 1999 (*supra*)). A recent report showed that IGF-1 stimulates $\alpha_2\delta$ -3 expression in cultured rat atrial myocytes. (Chu, P.-J., *J. Mol. Cell. Cardiology* (2003) 35:207-215.) The $\alpha_2\delta$ -4 subunit is distributed in certain cell types of the pituitary, adrenal gland, colon and fetal liver (Qin, *et al.*, 2002 (*supra*)).

[0009] A number of physiological roles have been proposed for the $\alpha_2\delta$ -2 subunit, including acting as a tumor suppressor gene, and a mutation in the mouse homolog, resulting in a truncated $\alpha_2\delta$ -2 has been identified as a contributing factor to the *ducky* epileptic phenotype (Gao, B., *et al.*, *J. Biol. Chem.* (2000) 275:12237-12242; Brodbeck, J., *et al.*, *J. Biol. Chem.* (2002) 277:7684-7693). The antiepileptic gabapentin binds to the $\alpha_2\delta$ -1 and -2 subunits, but not to $\alpha_2\delta$ -3 (Marais, E., *et al.*, *Molec. Pharmacol.* (2001) 59:1243-1248).

[0010] $\alpha_2\delta$ -1 increases the current density of calcium channels by increasing the amount of functional channel at the cell surface and enhances dihydropyridine binding to L-type channels and ω -conotoxin GVIA to N-type channels (Brust, P. F., *et al.*, *Neuropharmacology* (1993) 32:1089-1102; Felix, R., *et al.*, *J. Neurosci.* (1997) 17:6884-6891). $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 significantly enhance and modulate the Ca^{2+} current through a number of HVA and LVA channels (Klugbauer, *et al.* (1999) (*supra*); Gao, *et al.* (2000) (*supra*); Hobom, M., *et al.*, *Eur. J. Neurosci.* (2000) 12:1217-1226).

[0011] Recently, the molecular cloning of $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits from rat atria was reported. (Chu, P.-J., *et al.*, 2003 (*supra*)). Cloning of rat $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits from rat brain tissue has not been previously disclosed.

Disclosure of the Invention

[0012] The materials and methods of the present invention add to the repertoire of rat $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 calcium channel subunits previously known. Also provided are cell lines that express the novel calcium channels of the invention. These cells may be used for identifying compounds capable of acting as agonists or antagonists to the calcium channels.

[0013] Thus, in one aspect, the invention is directed to isolated nucleic acid molecules which contain a nucleotide sequence that encodes a protein having the amino acid sequence that is shown in SEQ. ID. NO:3, 6, 9 or 11, or a functional portion thereof. In another aspect, the invention relates to the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits themselves in isolated form that have an amino acid sequence that is shown in SEQ. ID. NO:3, 6, 9 or 11, or fragment of said sequence which retains the activity of this subunit. The invention is also directed to recombinant materials and methods for production of these proteins and displaying them on cells. When displayed on cells which also produce, contain and display at least an α_1 subunit, the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits of the invention in combination with the α_1 subunit provide active calcium ion channels which can be used to identify agonists and antagonists of calcium ion channel activity.

[0014] In other aspects, the invention is directed to nucleic acid probes that are specific for the particular $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunit mRNA's of the invention which permit the detection of expressed mRNA encoding the $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 protein. In addition, antibodies which are immunospecific for the particular $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 proteins of the invention can be used to map the distribution of the protein in cells and tissues.

Brief Description of the Drawings

[0015] The data in figures 1A and 1B show a comparison of the amino acid sequence of the rat brain $\alpha_2\delta$ -2 calcium channel subunit (SEQ. ID. NO:3) to that of the rat atria $\alpha_2\delta$ -2 calcium channel subunit (AF486277; SEQ. ID. NO:1).

[0016] The data in figures 2A and 2B show a comparison of the amino acid sequence of the rat brain $\alpha_2\delta$ -3 calcium channel subunit (SEQ. ID. NO:6) to that of the rat atria $\alpha_2\delta$ -3 calcium channel subunit (AF486278; SEQ. ID. NO:4).

[0017] The data in figures 3A and 3B show a comparison of the amino acid sequence of the rat brain $\alpha_2\delta$ -3 calcium channel subunit splice variant (SEQ. ID. NO:9) to that of the rat atria $\alpha_2\delta$ -3 calcium channel subunit (AF486278 SEQ. ID. NO:4).

[0018] The data in figure 4 show rat $\alpha_{1B} + \beta_{1b}$ Ca^{2+} channel current density when tested in the presence and absence of rat $\alpha_2\delta$ -2.

Modes of Carrying Out the Invention

[0019] The present invention provides amino acid sequences for novel rat calcium channel $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits, as well as nucleic acid sequences which encode these subunits. As described in the Background section above, it is understood that $\alpha_2\delta$ subunits alone do not mediate transport calcium across the cellular membrane; however, they increase the current density of calcium channels by increasing the amount of functional channel at the cell surface and enhancing binding of certain ligands. Typically, the $\alpha_2\delta$ subunits require the presence of an α_1 subunit, and are preferably expressed or assessed in the additional presence of β type subunits. If the α_1 subunit employed is from an L-type channel, the additional presence of a γ subunit is also desirable.

[0020] As described in the Background section above, there appear to be four specific types of this subunit, $\alpha_2\delta$ -1, $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 and $\alpha_2\delta$ -4. While in their native expression, each of these types may preferentially be associated with a particular class of calcium ion channel (*e.g.*, L, P/Q, N, T and the like), each of these subunits is functional when in association with any α_1 subunit with the optional addition of any type of β subunit. Although the text herein refers to $\alpha_2\delta$ because this is the protein encoded by a single gene, the encoded protein is hydrolyzed after translation into the α_2 and δ portions; the α_2 portion is mostly extracellular and is disulfide linked to the δ subunit which remains in the cell membrane. By "functional $\alpha_2\delta$ subunit" is meant the entire amino acid sequence encoded by the gene or any portion thereof which, when

associated with an α_1 subunit and optionally a β subunit enhances the density of current flow in the calcium ion channel thus created. Thus, it is understood that small numbers of amino acids, *e.g.*, 10-15 or 20 amino acids might be removed from each of the α_2 and/or δ portions while the remainder of the sequence retains functionality as described above. Thus, a “functional portion of the $\alpha_2\delta$ subunit” refers to these truncated forms.

[0021] A compound, such as a nucleic acid molecule or a protein is referred to as “isolated” when it is removed from its natural environment. It may or may not be pure. “Isolated” simply means that the molecule is in a context where it is not found in nature. For example, a nucleic acid comprising a particular nucleotide sequence is “isolated” when contained in a recombinant DNA molecule coupled to additional nucleotide sequences with which it is not normally associated. Similarly, the “protein” is isolated when it is not in the context of its native cell. “Isolated” $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits, for example, are frequently found in the context of a displayed calcium ion channel in a heterologous cell which has been modified to produce this protein.

[0022] Although in some instances, an α_1 subunit displayed at a cellular surface may be functional at some level in the absence of additional subunit types, the presence of the $\alpha_2\delta$ subunit greatly enhances the current density when the channel is activated. Accordingly, the production of $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 subunits for display on cells which also display at least α_1 subunits is important for use in screening assays to identify compounds that modulate the activity of the calcium ion channel.

[0023] The particular $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits that constitute the present invention were isolated and identified from rat brain. The amino acid sequence set forth as SEQ. ID. NO:3 is an $\alpha_2\delta$ -2 calcium channel subunit which is present in rat brain. SEQ. ID. NO:3 is 1,150 amino acids in length and has a molecular weight of 130 kDa. It is encoded by a nucleotide sequence containing 3,450 base pairs (bp) which is contained in the 4,993 bp sequence shown as SEQ. ID. NO:2. A full-length cDNA clone of 4,993 bp was constructed using a PCR fragment of 556 bp amplified from brain total RNA and a 4,507 bp fragment obtained from a brain cDNA library.

[0024] There is 95.5% and 90.5% nucleotide sequence identity of the rat $\alpha_2\delta$ -2 cDNA coding region compared to that of the mouse and human $\alpha_2\delta$ -2 subunits, respectively. A conceptual translation of the cDNA sequence shows that the rat $\alpha_2\delta$ -2 amino acid sequence shares 97.9% and 95.7% identity compared to the mouse and human $\alpha_2\delta$ -2 subunits, respectively.

[0025] It will be appreciated that polymorphic variations may be made or may exist in the $\alpha_2\text{d-2}$ and $\alpha_2\text{d-3}$ DNA of some individuals leading to minor deviations in the DNA or amino acids sequences from those shown which do not lead to any substantial alteration in the function of the calcium channel. Such variants, including variations that lead to substitutions of amino acids having similar properties are considered to be within the scope of the present invention.

[0026] An examination of the nucleotide sequence contained in SEQ. ID. NO:2 shows a Kozak consensus translation initiation sequence at the second start (AUG) codon. The encoded protein is predicted to have six N-glycosylated asparagines and a hydrophobicity profile similar to that of the rat $\alpha_2\text{d-1}$ subunit. There are 15 conserved cysteine residues in the rat $\alpha_2\text{d-2}$ protein, which correspond to those in the other mammalian $\alpha_2\text{d-2}$ proteins.

[0027] The $\alpha_2\text{d-2}$ subunit of SEQ. ID. NO:3 is 98.5% identical to the rat atrial $\alpha_2\text{d-2}$ subunit previously described (Accession Number AF486277, SEQ. ID. NO:1). See Figure 1 for comparative alignment of these sequences. There are several single point amino acid differences between the brain and atrial subunits. The atrial $\alpha_2\text{d-2}$ also contains an eight amino acid insert of LPISKCLKD (SEQ. ID. NO:7) at residues 665-672, suggesting that the brain and atrial subunits are splice variants. The $\alpha_2\text{d-2}$ subunits expressed in human and mouse brain also lack this insert.

[0028] The amino acid sequence set forth as SEQ ID NO:11 is an $\alpha_2\text{d-2}$ calcium channel variant which is present in rat brain. Without being bound by theory, this $\alpha_2\text{d-2}$ subunit appears to be a splice variant of the $\alpha_2\text{d-2}$ subunit of SEQ ID NO:3. The rat $\alpha_2\text{d-2}$ variant nucleotide sequence (SEQ ID NO:10) contains an insertion of 21 bases in comparison to the $\alpha_2\text{d-2}$ subunit nucleotide sequence SEQ ID NO:2. The insertion is found at nucleotides 1,994 to 2,014 in SEQ ID NO:10 (see nucleotides underlined and in bold in SEQ ID NO:10 sequence below).

[0029] The variant brain $\alpha_2\text{d-2}$ subunit contains an insertion of eight amino acids, LPISKCLKD (SEQ ID NO:7) at residues 665-672 in comparison to the $\alpha_2\text{d-2}$ subunit SEQ ID NO:3 (see amino acids underlined and in bold in SEQ ID NO:11 sequence below). This rat brain $\alpha_2\text{d-2}$ subunit variant protein sequence is 99.2% identical to the rat atrial $\alpha_2\text{d-2}$ subunit previously described (AF486277, SEQ ID NO:1).

[0030] This eight amino acid insertion is also found in the rat atrial $\alpha_2\text{d-2}$ subunit (see SEQ ID NO:1). The variant brain $\alpha_2\text{d-2}$ subunit is also expressed in human and mouse brain.

[0031] The amino acid sequence set forth as SEQ. ID. NO:6 is an $\alpha_2\text{d-3}$ calcium channel subunit which is present in rat brain. SEQ. ID. NO:6 is 1,091 amino acids in length and has a molecular weight of 123 kDa. It is encoded by a nucleotide sequence containing 3,273 bp which is contained in the 3,598 bp sequence shown in SEQ. ID. NO:5. A full-length cDNA clone of

3,598 bp was constructed using a PCR fragment of 1,618 bp amplified from rat brain total RNA and a 1,981 bp fragment isolated from a rat brain cDNA library.

[0032] An examination of the encoded protein in SEQ. ID. NO:6 shows a signal peptide cleavage site between residues 33 and 34, VRS-EQ. There are also four highly predictable N-glycosylation sites and 19 conserved cysteine residues between the rat, mouse and human protein sequences.

[0033] The rat brain $\alpha_2\text{d-3}$ DNA sequence has 86.5% and 95.9% identity to the human and mouse $\alpha_2\text{d-3}$ sequences, respectively. A conceptual translation of the cDNA sequence shows that the rat $\alpha_2\text{d-3}$ amino acid sequence shares 89.5% and 99.2% identity compared to the human and mouse, respectively.

[0034] The rat brain $\alpha_2\text{d-3}$ subunit cDNA sequence of SEQ. ID. NO:5 and protein sequence of SEQ. ID. NO:6 is 91.0% and 99.4%, respectively, identical to that of the rat atrial $\alpha_2\text{d-3}$ subunit previously described (AF486278, SEQ. ID. NO:4). See Figure 2 for alignment of these sequences. The human $\alpha_2\text{d-3}$ subunit is 997 amino acids while the rat brain $\alpha_2\text{d-3}$ sequence (SEQ. ID. NO:6) is 1,911 amino acids. The longer 5' region in the rat accounts for the 94 amino acid difference between human and rat.

[0035] The amino acid sequence set forth as SEQ. ID. NO:9 is an $\alpha_2\text{d-3}$ calcium channel subunit variant which is present in rat brain. Without being bound by theory, this $\alpha_2\text{d-3}$ subunit appears to be a splice variant of the full-length $\alpha_2\text{d-3}$ subunit. This variant $\alpha_2\text{d-3}$ sequence contains a 142 amino acid deletion of amino acid residues 817-958 in comparison to the full-length $\alpha_2\text{d-3}$ amino acid sequence (SEQ. ID. NO:6). This rat brain $\alpha_2\text{d-3}$ subunit variant protein sequence is 86% identical to the rat atrial $\alpha_2\text{d-3}$ subunit previously described (AF486278, SEQ. ID. NO:4). See Figure 3 for comparison.

[0036] Both the full-length and variant brain $\alpha_2\text{d-3}$ subunits contain an insertion of six amino acids, LPQAQK (SEQ ID NO:22), in comparison to the atrial $\alpha_2\text{d-3}$ subunit. Both human and mouse subunits contain the identical six residue sequence found in rat brain $\alpha_2\text{d-3}$. The full-length brain $\alpha_2\text{d-3}$ subunit also contains a single residue change in comparison to the atrial $\alpha_2\text{d-3}$ subunit.

[0037] When the $\alpha_2\text{d}$ protein is displayed on cells in the presence of or coexpressed with the α_1 subunit of any calcium ion channel and optionally as well, a β subunit, the resulting cells are useful in identifying compounds that modulate the activity of the channel. The nature of the channel is effectively determined by the nature of the α_1 subunit but, in all cases, the $\alpha_2\text{d}$ subunit enhances current flux so that a more accurate measurement can be made. A malfunction of

calcium ion channels is associated with a number of conditions; depending on the nature of the channel. For example, defects in calcium channels are associated with conditions including, but not limited to: epilepsy, migraine, ataxia, schizophrenia, hypertension, arrhythmia, angina, depression, small lung carcinoma, Lambert-Eaton syndrome.

[0038] Compounds identified that agonize or antagonize the various calcium ion channels are thus suitable drug candidates for treatment of these conditions. It is understood that not all agonists and antagonists thus identified will ultimately become successful drugs; however, the identification of a subpopulation of the millions of molecules that would otherwise be candidates represents a giant step toward development of a suitable drug.

[0039] While the required display of the calcium ion channels which include the $\alpha_2\delta$ subunits of the invention may be effected in a variety of animal cells, exemplary cells include *Xenopus* oocytes or mammalian cells such as human embryonic kidney (HEK 293) cells as described in PCT Publication No. WO 96/39512, incorporated herein by reference, and Ltk cells as described in U.S. Pat. No. 5,386,025, incorporated herein by reference. Transfection into host cells is accomplished by, for example, microinjection, lipofection, electroporation, calcium phosphate (glycerol shock) or particle-mediated gene transfer.

[0040] Mammalian cell lines stably expressing rat brain $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 calcium channels are, for example, prepared by transfecting an expression vector containing the $\alpha_2\delta$ -2 calcium channel cDNA or the $\alpha_2\delta$ -3 calcium channel cDNA into mammalian cells, such as HEK 293 cells, and selecting for cells containing the expression vector, for example, by selecting for the antibiotic resistance encoded by the expression vector, for example, pBK-RSV or pcDNA with a selectable marker (Invitrogen, San Diego, CA). The vectors are transfected into HEK 293 cells by calcium phosphate coprecipitation or lipofection or electroporation or any other method according to well known procedures (Methods in Enzymology, Volume 185, "Gene Expression Technology" (1990) Edited by Goeddel, D. V.). The rat brain $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 calcium channel subunit cDNA expression vector may be transfected alone, or in combination with other rat, human or other mammalian or other animal calcium channel subunit cDNA's, such as the α_{1B} and β_{1b} subunit cDNA's, either in a similar expression vector or other type of vector using different selectable markers. Transfected cells are typically incubated for 4-16 hours under transfection conditions at 37°C, 5% CO₂, then placed in nonselective medium for an additional 24 hours. The cells are trypsinized and plated at low density in selective medium containing, for example, Geneticin (G418) between 600 to 800 µg/ml or Zeocin between 25-200 µg/ml, depending upon the particular vector. After 10-16 days in selective medium, cells that are

resistant to G418 or zeocin grow as visible colonies and isolated colonies are harvested by the pipet technique or using standard cloning rings. Isolated cell colonies are then expanded to make frozen stocks of cells and to determine the level of rat $\alpha_2\text{d-2}$ or $\alpha_2\text{d-3}$ subunit expression. Southern blotting can be used to detect the integration of the subunit nucleotide sequence into the cell genome, the presence of the plasmid episomally, and the number of copies present. Rat $\alpha_2\text{d-2}$ and $\alpha_2\text{d-3}$ expression levels for the cell lines are determined using standard gene expression methods such as Northern blotting, RNase protection, reverse-transcriptase PCR, and Western blotting.

[0041] The functional detection of calcium channels containing the rat $\alpha_2\text{d-2}$ or $\alpha_2\text{d-3}$ subunit of the invention in stably transfected cells can be examined electrophysiologically, such as by whole cell patch clamp or single channel analysis, as described herein, for example. Other means of detecting functional calcium channels include the use of radiolabeled ^{45}Ca uptake, or fluorescence spectroscopy using calcium sensitive dyes, such as FURA-2.

[0042] The resulting cell lines expressing functional calcium channels comprising the $\alpha_2\delta$ subunit of the invention and at least an α_1 subunit can then be used as test compounds for pharmacological activity with respect to these calcium channels as set forth above.

[0043] Compounds to be tested as agonists or antagonists of the calcium channels are combined with cells that are stably or transiently transformed with a cDNA sequence encoding the rat $\alpha_2\text{d-2}$ or the $\alpha_2\text{d-3}$ subunit of the invention with α_1 and, optionally, β subunits and monitored for pharmacological activity, if any, with respect to these calcium channels. Thus, the cell lines are useful for screening compounds for pharmaceutical utility.

[0044] Such screening can be carried out using several available methods for evaluation of the interaction, if any, between the test compound and the calcium channel. One such method involves the binding of radiolabeled agents that interact with the calcium channel and subsequent analysis of equilibrium binding measurements including, but not limited to, on rates, off rates, K_d values and competitive binding by other molecules. Another method involves screening for the effects of compounds by electrophysiological assay whereby individual cells are impaled with a microelectrode and currents through the calcium channel are recorded before and after application of the compound of interest. Another method, high-throughput spectrophotometric assay, utilizes loading the cell lines with a fluorescent dye sensitive to intracellular calcium concentration and subsequent examination of the effects of compounds on the ability of depolarization by potassium chloride or other means to alter intracellular calcium levels.

[0045] Compounds that are found to modulate the calcium ion channels, wherein the rat $\alpha_2\delta$ -2 or the $\alpha_2\delta$ -3 subunit of the invention is used to enhance the signal, and thereby increase the accuracy and reproducibility of results, are useful in treating conditions associated with defects in performance of these channels. The nature of these conditions depends on the type of calcium ion channel involved; but since the $\alpha_2\delta$ subunit of the invention enhances the signal for all types of calcium ion channels, it is useful in detecting compounds for a wide variety of conditions. Defects in calcium channels are associated with conditions including, but not limited to: epilepsy, migraine, ataxia, schizophrenia, hypertension, arrhythmia, angina, depression, small lung carcinoma, Lambert-Eaton syndrome.

Detection of Calcium Ion Channel Expression

[0046] Expression of the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits of the invention, and of calcium ion channels that contain the $\alpha_2\delta$ subunits of the invention, can be detected at the mRNA or protein level. Accordingly, in some embodiments, methods of the invention involve assaying biological samples for the presence, absence and/or level of the $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 subunit probe target of the invention, such as rat brain $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 subunit RNA and/or rat brain $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 subunit polypeptide.

[0047] It will be readily apparent upon reading of the present specification that the expression detection assays can be conducted as, or modified to be conducted as, *in vitro* or *in vivo* assays, and may be either cell-free (*e.g.*, *in vitro* binding assays using polynucleotides isolated from or produced from nucleic acid of a biological sample) or cell-based (*e.g.*, screening of whole cells for expressing the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits of the invention). In general, all assays are conducted under conditions, and for a period of time, sufficient to allow for specific binding of a rat brain $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 subunit-specific probe (*e.g.*, nucleic acid probe, antibody probe) to a rat brain $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 subunit probe target, *e.g.*, to provide for detection of rat brain $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 subunit probe target at a detectable level above background. The assays can include various positive and/or negative controls, the nature of which will be readily apparent to the ordinarily skilled artisan upon reading the present specification.

[0048] Any suitable qualitative or quantitative methods known in the art for detecting specific $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 mRNA's can be used to detect or quantitate expression of the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 mRNA of the invention. For example, rat brain $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 mRNA in cells can be measured by various techniques known in the art including, but not limited to, S1 nuclease analysis, ribonuclease protection assay, primer extension assay, RNA blot analysis (*e.g.*,

northern and/or slot blot hybridization) and amplification techniques including reverse transcriptase-PCR (RT-PCR). In addition, expression can be assessed in histological assays, for example the $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 RNA of the invention can be detected by *in situ* hybridization in tissue sections, using methods that detect single base pair differences between hybridizing nucleic acid and other methods well known in the art.

[0049] Typically northern blot of total mRNA or fractionated RNA or RT-PCR are employed. The northern blot or RT-PCR product is probed under conditions of high stringency with a fragment of SEQ. ID. NO:2, 5, 8 or 10, or the complement thereof, whichever is appropriate. Typically, the probes contain at least 12 consecutive nucleotides derived from SEQ. ID. NO:2, 5, 8 or 10, or the complement thereof. Conditions of high stringency are defined as wash conditions of 1 x SSC, 0.1 SDS, and 60°C. When mRNA encoding the $\alpha_2\delta$ subunits of the invention is present, its presence and amount can thus be detected.

[0050] To differentially detect the rat brain $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 mRNA's of the invention from their rat atrial counterparts (and likely other $\alpha_2\delta$ mRNA's), probes containing nucleotide sequences which encode the polypeptide regions which differ between the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 proteins of the invention and those known in the art may be used. For example, to specifically detect rat brain $\alpha_2\delta$ -2 mRNA from rat atrial $\alpha_2\delta$ -2 mRNA, a probe can be used which contains the nucleotide sequence that flanks and includes base pairs 2,015-2,021 of SEQ ID NO:2, for example, a probe containing base pairs 2,011-2,023 of SEQ. ID. NO:2. To specifically detect rat brain $\alpha_2\delta$ -2 variant mRNA from rat brain $\alpha_2\delta$ -2 mRNA for SEQ ID NO:3 and from rat atrial $\alpha_2\delta$ -2 mRNA, a probe can be used which contains the nucleotide sequence that flanks and includes base pairs 1,994 to 2,014 of SEQ ID NO:10 in conjunction a second probe which detects the sequence differences between brain and atrial $\alpha_2\delta$ -2, as indicated, for example, in Figures 1A and 1B. To specifically detect rat brain $\alpha_2\delta$ -3 mRNA from rat atrial $\alpha_2\delta$ -3 mRNA, a probe can be used which contains the nucleotide sequence that flanks and includes base pairs 1,381 to 1,398 of SEQ ID NO:5. To differentiate between rat brain $\alpha_2\delta$ -3 full-length mRNA and $\alpha_2\delta$ -3 variant mRNA, a probe can be used which contains portions of the nucleotide sequence contained only in the $\alpha_2\delta$ -3 full-length mRNA SEQ. ID. NO:5 and not is SEQ. ID. NO:8, for example, nucleotide sequences between about base pair 2,449 and about base pair 2,874 of SEQ. ID. NO:5. Alternatively, a probe to specifically detect the $\alpha_2\delta$ -3 variant mRNA from the $\alpha_2\delta$ -3 full-length mRNA can be generated to include nucleotide sequence that flanks and includes base pairs 2,448 and 2,449 of SEQ. ID. NO:9, for example, nucleotide sequences from about base

pair 2,442 to about 2,455 of SEQ ID NO:9. The mRNA's of the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits of the invention can also be distinguished from each other by length.

[0051] Nucleic acid probes can be prepared using routine methods, including automated oligonucleotide synthetic methods. For use of such probes, the biological sample to be analyzed may be treated, if desired, to extract the RNA contained therein. The resulting RNA from the sample may be subjected to gel electrophoresis or other size separation techniques; alternatively, the RNA sample may be dot blotted without size separation. The probes are usually labeled with a detectable label. Suitable labels, and methods for labeling probes are known in the art, and include, for example, radioactive labels incorporated by nick translation or kinasing, biotin, fluorescent probes, and chemiluminescent probes. The RNA extracted from the sample is then treated with the labeled probe under hybridization conditions of suitable stringencies.

[0052] In addition to detecting mRNA production, the probes of the invention, as described above, can be used to recover nucleotide sequences encoding $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits from other animals. The probes may be used with respect to cDNA libraries or genomic libraries derived from other species, and are tested for hybridization under the high stringency conditions described above.

[0053] In one embodiment, the invention features methods for detecting expression of rat brain $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 subunits through detection of the $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 polypeptides in a biological sample. Polypeptide-based detection can be accomplished by use of an antibody (including antigen-binding antibody fragments) or a receptor (including ligand-binding receptor fragments) that specifically binds the target $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 polypeptides (*e.g.*, an anti-rat brain $\alpha_2\delta$ -2 polypeptide antibody and anti-rat brain $\alpha_2\delta$ -3 polypeptide antibody). For example, the presence of target $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3 polypeptides in a sample can be determined using a target $\alpha_2\delta$ -2 and/or $\alpha_2\delta$ -3-specific probe using various techniques known in the art including, but not limited to, quantitative immunoassays, such as, radioimmunoassay, immunofluorescent assay, enzyme immunoassay, chemiluminescent assay, ELISA, western blot assay, immunocytochemistry assay or immunohistochemistry assay.

[0054] For detection at the protein level, it is convenient to produce antibodies to $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 proteins of the invention. The antibodies are immunospecific to these proteins if they immunoreact detectably more strongly to the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 subunits of the invention as compared to other proteins, including $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 proteins of the prior art. The antibodies may be polyclonal, monoclonal, single-chain recombinant, and the like. Methods for preparation of such antibodies, including antibodies designed to be compatible with individual

species such as humanized antibodies are well known. It is also understood that the term "antibodies" includes immunospecific fragments thereof, such as F_{ab} , F_{ab}' , and the like. As mentioned above, single-chain F_v antibodies also represent useful fragments. The rat brain $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 full length and $\alpha_2\delta$ -3 variant proteins can also be distinguished from each other by differences in molecular weight.

[0055] To differentially detect the rat brain $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 proteins of the invention from their rat atrial counterparts (and likely other $\alpha_2\delta$ proteins), antibodies specific to the polypeptide regions in which the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 proteins of the invention differ from those known in the art can be produced. For example, to specifically detect rat brain $\alpha_2\delta$ -2 protein, an antibody can be produced to the polypeptide region that flanks and includes amino acids 664-666, such as a polypeptide comprising the amino acid sequence from about 660 to about 670 of SEQ. ID. NO:3. To specifically detect rat brain $\alpha_2\delta$ -3 protein from rat atrial $\alpha_2\delta$ -3 protein, an antibody can be produced to the polypeptide region that flanks and includes amino acids 461 to 466, such as a polypeptide comprising the amino acid sequence from about 457 to about 470 of SEQ. ID. NO:6.

[0056] To differentiate between rat brain $\alpha_2\delta$ -3 full-length protein (SEQ. ID. NO:6) and the $\alpha_2\delta$ -3 variant (SEQ. ID. NO:9), antibodies can be produced directed to a portion of the full-length protein missing from the variant, for example from about amino acid 817 to about amino acid 958 of SEQ. ID. NO:6. Such an antibody would likely also distinguish between the rat atrial $\alpha_2\delta$ -3 full-length protein (SEQ. ID. NO:4) and the rat brain $\alpha_2\delta$ -3 variant (SEQ. ID. NO:9). In addition, or alternatively, to specifically detect the rat brain $\alpha_2\delta$ -3 variant (SEQ. ID. NO:9), antibodies can be generated directed to a polypeptide spanning the portion missing relative to the full-length protein. For example, an antibody specific for a polypeptide that includes amino acids flanking amino acids 816 and 817 of SEQ. ID. NO:9, such as a polypeptide comprising the amino acid sequence from about 810 to about 824 of SEQ. ID. NO:9, would allow specific detection of the $\alpha_2\delta$ -3 variant polypeptide.

[0057] A combination of antibodies may also be used to identify and/or quantitate the $\alpha_2\delta$ -3 variant from the full length $\alpha_2\delta$ -3 polypeptide. In such a case, one antibody which recognizes the shared portion of the two polypeptides, *i.e.*, a section of the N-terminal portion from amino acid 1 to about amino acid 817, and the other antibody which recognizes the portion of the full-length protein missing from the variant, for example from about amino acid 817 to about amino acid 958 of SEQ. ID. NO:6, can be used. Detection of differences in binding of the two antibodies could be used to distinguish rat brain $\alpha_2\delta$ -3 full length from rat brain $\alpha_2\delta$ -3 variant.

[0058] These antibodies can be used to detect the production of any calcium channel which includes the $\alpha_2\delta$ calcium ion channels on histological sections or tissue extracts. Expression and display on recombinant cells can also be detected using these antibodies. Standard methods for labeling and detecting the antibody complexes are employed.

[0059] Complexes formed comprising the $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 polypeptides of the invention and the anti- $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 subunit antibody are detected by any of a number of known techniques, depending on the format. The antibody in the immunoassays for detection of the $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 polypeptides of the invention may be provided on a support (*e.g.*, solid or semi-solid); alternatively, the polypeptides in the sample can be immobilized on a support. Examples of supports that can be used are nitrocellulose (*e.g.*, in membrane or microtiter well form), polyvinyl chloride (*e.g.*, in sheets or microtiter wells), polystyrene latex (*e.g.*, in beads or microtiter plates), polyvinylidene fluoride, diazotized paper, nylon membranes, activated beads, and Protein A beads. Bead-based supports are generally more useful for immobilization of the antibody in the assay.

[0060] In one embodiment, the biological sample contains cells (*i.e.*, whole cells) and detection is by reacting the sample with labeled antibodies, performed in accordance with conventional methods. In general, antibodies that specifically bind a $\alpha_2\delta$ -2 or $\alpha_2\delta$ -3 polypeptide of the invention are added to a sample, and incubated for a period of time sufficient to allow binding to the epitope, usually at least about 10 minutes. The antibody can be detectably labeled for direct detection (*e.g.*, using radioisotopes, enzymes, fluorescers, chemilumescers, and the like), or can be used in conjunction with a second stage antibody or reagent to detect binding (*e.g.*, biotin with horseradish peroxidase-conjugated avidin, a secondary antibody conjugated to a fluorescent compound, *e.g.*, fluorescein, rhodamine, Texas red, and others). The absence or presence of antibody binding can be determined by various methods, including, but not limited to, flow cytometry of dissociated cells, microscopy, radiography, and scintillation counting. Any suitable alternative methods of qualitative or quantitative detection of levels or amounts of differentially expressed polypeptide can be used, for example ELISA, Western blot, immunoprecipitation, radioimmunoassay, and the like.

[0061] The following examples are offered to illustrate but not to limit the invention.

Example 1

[0062] Novel $\alpha_2\delta$ -2 subunits of the invention were identified using RT-PCR of rat brain total RNA and screening of a rat brain cDNA library. Screening of the cDNA library followed

standard methods and included infecting bacteria with recombinant lambda phage and immobilizing lambda DNA on to nylon filters (Hybond-N, Amersham Pharmacia biotech).

[0063] A rat α_2 d-2 cDNA probe of 313 bp (1,368-1,680) was amplified from rat brain total RNA using One-Step RT-PCR (Superscript, Invitrogen) with primers designed to conserved regions of the mouse and human α_2 d-2 genes as follows:

F1368, 5'-AGCCATCCGCATCAACACACAG-3' (SEQ ID NO:12);

R1680, 5'-AGCAACACGTAGCCGTTTCAGGTC-3' (SEQ ID NO:13).

[0064] The resulting probe was radiolabeled with [$a^{32}P$] dCTP using the Multi-Prime DNA labeling System (Amersham) and the free $a^{32}P$ removed using a Centri Spin-20 column (Princeton Separations). Approximately 300,000 plaque forming units (pfus) were screened with the radiolabeled probe using moderate hybridization (55°C) in Church and Gilbert's solution and moderate to high wash conditions (55°C, 0.2 X SSC/ 0.1% SDS). Bacteriophage that hybridized to the rat α_2 d-2 radiolabeled probe were detected by exposing the membranes to autoradiography. Positive clones were purified by sequential rounds of screening and the phagemid cDNA (pBluescript SK+) isolated by *in vivo* excision using the ExAssist helper phage with SOLR Strain *E. coli* according to the manufacturer's instructions (Stratagene).

[0065] Ten positive clones were sequenced and found to contain cDNA fragments of the rat α_2 d-2 subunit (4.0 Kb >). Two clones were completely sequenced of which one clone, rat library clone 11-1-2, contained a sequence of 4,507 base pairs (bp). This clone was missing 487 bp of the 5' open reading frame (ORF). In order to obtain the 5' end of the rat α_2 d-2 cDNA, antisense primers were made to the rat α_2 d-2 sequence and sense primers were designed to consensus sequence of the human and mouse subunits.

[0066] Primer 591R: 5'-GCTCCTCGATGAAGTCCAGCCTTA-3' (SEQ ID NO:14), was used in the reverse transcriptase (RT) reaction of rat brain total RNA (60°C, 90 min). A 561 bp 5' fragment of the rat α_2 d-2 mRNA, including 24 base pairs of the 5' untranslated sequence, was amplified using PCR from the RT reaction using Hot Star Taq plus Q-solution (Qiagen) with a sense primer designed to the human and mouse 5' UTR sequence:

F-24: 5'-CGGCGCCGCATCTTGAATGGAAAC-3' (SEQ ID NO:15),

and an antisense primer to the rat α_2 d-2 library clone.

[0067] The α_2 d-2 PCR fragment was subsequently inserted into pGEM-T (Promega). In order to make a full-length rat α_2 d-2 cDNA, subunit primers were designed with the restriction enzyme sites, Hind III (F-5: 5'-GCGAAGCTTGAACATGGCGGTGC-3' (SEQ ID NO:16)),

which removes the first AUG, and BamH I

(557R: 5'-TGGATCCCCTCTCCATATCCTCACTC-3' (SEQ ID NO:17)).

[0068] The PCR was done using Pfu (Stratagene) and the cDNA products were cloned into pGEM-T. The full-length rat $\alpha_2\delta$ -2 sequence was constructed in the mammalian expression vector pBK-RSV (-lac p) using a three way ligation of the Hind III/BamH I 5' PCR fragment and the BamH I/Xba I region of the rat library clone 11-1-2.

[0069] The full-length rat brain $\alpha_2\delta$ -2 cDNA clone has the nucleic acid SEQ. ID. NO:2:
Full-length rat brain $\alpha_2\delta$ -2 calcium channel subunit nucleotide sequence (SEQ. ID. NO:2)

```

1   CGGCGCCGCA TCTTGAATGG AAACATGCGC GTGCCGGCTC GGACCTGCGG CGCTTCTTGG
61  CCCGGCCCGG TCGGGACCGC TCGCCCTGG CCCGGTCGG GTCCCCGGCC CTGCCCTGAC
121 CCCCGGGGCC CAGCGTCCGG GCGCGCACGC CCGCTCTTGC TACTGCTGCC GCCTCTGCTG
181 CTTTTACCGC TGCTCACC GC CCGCGCGCC TCTGCCTACA GCTTCCCCCA GCAGCACACG
241 ATGCAGCACT GGGCCCGGCG CCTGGAGCAG GAGATTGACG GTGTGATGCG GATTTTTGGA
301 GGCGTGCAGC AGCTCCGAGA GATCTACAAG GACAATCGGA ACCTGTTTGA TGTGCAGGAG
361 AATGAACCAC AGAAACTAGT GGAGAAGGTG GCAGGGGACA TTGAGAGCCT GCTGGACAGA
421 AAGGTCCAGG CCTTGAAGAG ACTGGCTGAC GCTGCAGAGA ATTTCCAGAA AGCCCACCGC
481 TGGCAAGACA ACATCAAGGA GGAAGACATC ATGTACTATG ACGCCAAGGC TGACGCCGAG
541 CTGGATGATC CTGAGAGTGA GGATATGGAG AGGGGATCCA AGACCAGCGC CTTAAGGCTG
601 GACTTCATCG AGGAGCCAAA CTTCAAGAAC AAAGTCAACT ATTCATACAC GCGGTGCAG
661 ATCCCCACAG ATATCTACAA AGGCTCTACC GTCATCCTCA ATGAGCTTAA CTGGACGGAG
721 CCCCTGGAGA ATGTCTTCAT TGAGAACCGT AGGCAAGACC CTACACTGTT GTGGCAAGTC
781 TTCGGCAGTG CCACGGGAGT CACCCGCTAT TACCCAGCCA CACCGTGGCG AGCCCCCAAG
841 AAGATTGACC TGTACGATGT CAGAAGACGA CCCTGGTATA TACAGGGGGC CTCATCACCC
901 AAGGACATGG TCATCATTGT GGATGTGAGT GGCAGTGTGA GCGGCCTGAC GCTGAAGCTG
961 ATGAAGACGT CTGTCTGTGA GATGCTAGAC ACACTCTCCG ATGATGACTA TGTGAATGTG
1021 GCCTCATTCA ACGAGAAGGC GCAGCCTGTG TCTTGCTTCA CACACCTGGT GCAGGCCAAT
1081 GTGCGGAACA AGAAGGTGTT CAAGGAAGCT GTGCAGGGCA TGGTGGCCAA GGGCACCACA
1141 GGCTACAAGG CTGGCTTTGA GTATGCCTTT GACCAGCTAC AGAATTCCAA CATCACCCGT
1201 GCTAACTGCA ATAAGATGAT CATGATGTTC ACGGACGGGG GAGAGGATCG CGTGCAGGAC
1261 GTCTTTGAAA AGTATAATTG GCCCAATCGG ACGGTACGG TCTTCACGTT CTCCGTAGGA
1321 CAGCATAACT ATGATGTCAC ACCCTGCAG TGGATGGCTT GACTAACAAC AGGTTACTAT
1381 TTTGAGATCC CTTCCATCGG AGCCATCCGC ATCAACACAC AGGAATACCT GGATGTGCTG
1441 GGTAGGCCCA TGGTCTTGGC AGGCAAGGAG GCCAAGCAAG TGCAATGGAC AAACGTGTAT
1501 GAAGATGCGC TGGGCTGGG GTTGGTGGTA ACAGGGACTC TCCCTGTTTT CAACCTGACA
1561 CAGGATGGCC CTGGGGAAAA GAAGAACCAG CTAATCCTGG GTGTGATGGG CATAGATGTG
1621 GCCCTGAATG ACATCAAAAG GCTGACTCCC AACTACACAC TTGGCGCCAA TGGCTACGTG
1681 TTCGCCATCG ACCTGAATGG CTATGTGTTG CTACATCCCA ATCTCAAGCC CCAGACTACC
1741 AACTTCCGGG AGCCTGTGAC CTTGGACTTC CTGGATGCAG AGCTGGAAGA TGAGAACAAG
1801 GAGGAGATCC GTCGTAGTAT GATTGACGAA GACAAAGGCC ACAAGCAGAT CAGAACCTTG
1861 GTCAAATCCC TGGATGAGAG GTACATAGAT GAAGTGATTC GGAACACAC CTGGGTGCCT
1921 ATAAGGAGTA CCAACTACAG CCTGGGGCTG GTGCTCCAC CCTACAGCAC CTACTACCTC
1981 CAAGCCAACC TCAGCGACCA GATCCTGCAG GTCAAGTATT TTGAGTTTCT GCTCCCCAGC
2041 AGCTTTGAGT CTGAAGGACA TGTTTTCAAT GCTCCAGAG AGTATTGCAA GGATTTGAAT
2101 GCCTCAGACA ACAACACCGA GTTCTGAAA AACTTCATTG AGCTCATGGA GAAAGTGACT
2161 CCGGACTCCA AGCAGTGCAA TAACTTCCTT CTGCATAACT TGATTTTGA CACGGGCATT
2221 ACGCAGCAGT TAGTGGAACG CGTCTGGCGG GACCAAGATC TCAACAGTA CAGCCTGCTA
2281 GCTGTATTTG CTGCCACTGA CCGTGACGTC ACACGTGTCT TCCCGAACAA GGCAGCTGAA
2341 GACTGGACAG AAAACCCTGA ACCCTTCAAT GCCAGCTTCT ACCGCCGAG CCTGGATAAC
2401 CGTGGTTATA TCTTCAAGCC CCCGCACCAG GACTCCCTGT TAAGGCCACT GGAGCTGGAG
2461 AATGACACAG TAGGCGTCCT CGTCAGCACA GCTGTAGAGC TCAGTCTGGG TCGCCGACA
2521 CTGAGGCCAG CAGTGGTGGG TGTCAACTG GACCTAGAGG CTTGGGCTGA AAAGTTCAAG
2581 GTGCTTGCCA GCAACCGTAC CCATCAGGAC CAACCTCAGA AGCAGTGCGG CCCAGCAGC

```

```

2641  CACTGTGAGA TGGACTGCGA GGTAACAAC GAGGACCTAC TCTGTGTCCT CATTGATGAC
2701  GGGGGATTCC TGGTGCTGTC AAACCAGAAC CACCAGTGGG ACCAGGTTGG CAGATTCTTC
2761  AGTGAGGTGG ATGCCAACCT GATGCTGGCA CTGTACAATA ACTCCTTCTA CACCAGAAAG
2821  GAGTCCTATG ACTATCAGGC AGCTTGCGCC CCTCAGCCTC CTGGCAACCT GGGTGTGCA
2881  CCCAGGGGTG TCTTTGTGCC CACCATTGCA GATTTCTTAA ACTTGGCCTG GTGGACCTCT
2941  GCTGCCGCCT GGTCTTTATT CCAGCAACTA CTTTATGGTC TCATCTATCA CAGCTGGTTC
3001  CAGGCAGACC CGGCAGAAGC CGAGGGCAGC CCCGAGACGC GCGAGAGCAG CTGCGTCATG
3061  AAACAAACCC AGTACTACTT CGGCTCGGTG AACGCGTCCT ATAACGCCAT CATCGACTGC
3121  GGAAACTGCA GCAGGCTGTT CCACGCGCAG AGACTGACCA ACACCAACCT TCTGTTTCGTG
3181  GTGGCGGAGA AGCCGCTGTG CAGCCAGTGC GAGGTCGGCC GGCTGCTACA AAAGGAGACA
3241  CACTGCCCAG CGGACGGCCC GGAGCAGTGT GAGCTGGTGC AGAGACCGCG ATACCGAAGA
3301  GGCCCGCACA TCTGTTTTGA CTACAATGCG ACGGAAGATA CCTCAGACTG TGGCCGCGGC
3361  ACGTCCTTCC CTCCGTCGCT GGGCGTCTTG GTTTCCTGCG AGCTGTTGCT CCTCTAGGC
3421  CTGCCACCTC GGCCGCAGCC TCAAATCCAT TCCTTCGCTG CCTCTCGCCG CCTCTGAACT
3481  ACCCACACAC ACACATCATA GCCCCACCC CCACCCCGCC TTGGCCTCCT AGCCTTTTCG
3541  CTCACCCTCC CATGCCACAT TCCCCAATCT AGATCCTTGG CCACTCTCTC CTGAAGGAAC
3601  TGGGCCCCCTT CCCCAGAGCC TGTGCCCTGG GGCAGGGGAG CCAAAGTAAG GTGCCATGGT
3661  GTTTGGCATT CAAGATTTAT CTCACCCTTG AACTGTCCAA GTGCCACAG TCCCTGGACT
3721  CACCCCTGTG GTTGGGACAG GAGGCCACTA GTACCGATGC CAAACCAGGC CTCCACCAAC
3781  CCACCTGCCT GGAGATTTTC TCTATGTAGG CAACCCTGCC ACTGCTGGGC GCCTCTAACT
3841  GGCCCTTTGC CCCACCCAGG CCCAACTTA CCTTCTCTGG GGAAAAAACT AGGAGAGATG
3901  GNTAGTGGTG AGAGAGATTC TGGGGGCACC CCTTCCCCAT AGCCTCGGGC CGTTCAGGC
3961  TACACCACAA ACCCACACCT CGGCTTGCGAG GTATCAGGAC AGCCTCACGA TGACATCAGC
4021  TTAGGCACAC CCCACAGACA CCTGGACCTC AGAGAGCAGA AACTGGACTC TCACTAGACA
4081  TGCCCAGAGG GGAACACACA AACAGACACA CACCATGGGG GACCCACAAA GCCTTACACA
4141  GGGCGAGAGG TCAGTGAAGG GGCTGACCTG TGTGTTCCCT CTCCGCTCAC CTCTGCCTCC
4201  ACTCTGAGAT GCAGCCTGGC AGGCCCTCCC ATCTCTAGAA CTGAATGTCA GACCGTGCCA
4261  AATGCTAGGG GAAGGCCTCT GTTTCGCCCC TAGCCACCAG TGTCCCCAAA TGCCCCTCAC
4321  CCTGCCAGGT GCTCATTGTA ACCATTGCTC ACTAGTGTCA GGCCCTAGT AGGACCACAT
4381  GCTACTGCCT GAACCCCTTT GGCAGAAGAA CCCCAGCAGA CATTGTACTT TGCCTTAGCA
4441  GGGGTGACTT GGTCTCTCCT GGCTGGGCCA TCCCATCCCC AATCTGGTTC TTACATACTC
4501  AGGCCTAATT CCCTCTTCAC ACACACACAC ACACACACAC ACACACACAC ACACACACAG
4561  TCCCTGCCCC TAGGAGGCCA TATTGCCCTT CCCTTGCTGA ACACACACTT GCACCAAGCA
4621  CATGTGTAGT CAACCATACT GCACACACAG AGGCTGGGCC TGGGACACAT CTCTTCACAC
4681  CATTCACTCT GTCATTTCTC CCAAAGGCAT CGTAACCTGG GGGCCAGGCG GGGACTGAGG
4741  GCAGGGTGGG GGGGTGTGTG GCCATGAGGC TCAGATGGAC TGGGAGGAGG GTGGGAGGGT
4801  GGTACATTAA TTAATGGCTC CGTTAATTAA TGTCATGTTG CGTGTGCTT TCTCAGTGTG
4861  TGTATGGTCC ATGCCCAATG CTGGTGGCAG GGTGGGTGTC CATGATGTGT GCCCAGCCTG
4921  GATGTCAGCT GTGTCCTGTG GGGCGTGTG TGTAAGTGTG GTGTAGTCAG GTGCTCAACG
4981  GAGAATACAA ACG

```

and the deduced amino acid sequence of rat brain $\alpha 2\delta$ -2 cDNA is SEQ. ID. NO:3.

[0070] Translated rat brain $\alpha 2\delta$ -2 amino acid sequence (SEQ. ID. NO:3):

```

1  MAVPARTCGA SWPGPVRTAR PWPGRGPRPC PDPRGPASGP ARPLLLLLLPP
51  LLLLPLLTAP GASAYSFPQQ HTMQHWARRL EQEIDGVMRI FGGVQQLREI
101 YKDNRNLFVD QENEPQKLVE KVAGDIESLL DRKVQALKRL ADAAENFQKA
151 HRWQDNIKEE DIMYYDAKAD AELDDPESED MERGSKTSAL RLD FIEEPNF
201 KNKVNYSYTA VQIPTDIYKG STVILNELNW TEALENVFIE NRRQDPTLLW
251 QVFGSATGVT RYYPATPWRA PKKIDLYDVR RRPWYIQGAS SPKDMVIIVD
301 VSGSVSGLTL KLMKTSVCEM LDTLSDDDYV NVASFNEKAQ PVSCFTHLVQ
351 ANVRNKKVFK EAVQGMVAKG TTGYKAGFEY AFDQLQNSNI TRANCNKMIM
401 MFTDGGEDRV QDVFKEYNWP NRTVRVFTFS VGQHNYDVTP LQWMACTNKG
451 YYFEIPSIGA IRINTQEYLD VLGRPMVLG KDAKQVQWTN VYEDALGLGL
501 VVTGTLPVFN LTQDGPGEKK NQLILGVMGI DVALNDIKRL TPNYTLGANG
551 YVFAIDLNGY VLLHPNLKPQ TTNFREPVT LDFLDAELEDE NKEEIRRSMI
601 DEDKGHKQIR TLVKSLDERY IDEVIRNYTW VPIRSTNYS LGLVLPYSTY
651 YLQANLSDQI LQVKYFEFL PSSFESEGHV FIAPREYCKD LNASDNNTF

```

```

701 LKNFIELMEK VTPDSKQCNN FLLHNLILDT GITQQLVERV WRDQDLNTYS
751 LLAVFAATDG AVTRVFPNKA AEDWTENPEP FNASFYRRSL DNRGYIFKPP
801 HQDSLRLRPLE LENDTVGVLV STAVELSLGR RTLRAVVG V KLDLEAWAEK
851 FKVLASNRTH QDQPQKQCGP SSHCEMDCEV NNEDLLCVLI DDGGFLVLSN
901 QNHQWDQVGR FFSEVDANLM LALYNNSFYT RKESYDYQAA CAPQPPGNLG
951 AAPRGV FVPT IADFLNLAWW TSAAAWSLFQ QLLYGLIYHS WFQADPAEAE
1001 GSPETRESSC VMKQTQYYFG SVNASYNAIL DCGNCSRLFH AQRLTNTNLL
1051 FVVAEKPLCS QCEVGRLLQK ETHCPADGPE QCELVQRPRY RRGPHICFDY
1101 NATEDTSDCG RGTSFPPLSG VLVSLQLLLL LGLPPRPQPQ IHSFAASRRL
1151 *

```

[0071] The rat brain $\alpha_2\delta$ -2 variant cDNA clone was also identified and has SEQ ID NO:10:

Rat brain $\alpha_2\delta$ -2 variant calcium channel subunit nucleotide sequence (SEQ. ID. NO:10)

```

1 ATGGCGGTGC CGGCTCGGAC CTGCGGCGCT TCTTGGCCCG GCCCGGTGCG GACCGCTCGC
CCCTGGCCCG GTCGCGGTCC
81 CCGGCCCTGC CCTGACCCCC GGGGCCAGC GTCCGGGCCC GCACGCCCGC TCTTGCTACT
GCTGCCGCTT CTGCTGCTTT
161 TACCGCTGCT CACCGCCCCC GCGCCTCTG CCTACAGCTT CCCCAGCAG CACACGATGC
AGCACTGGGC CCGGCGCCTG
241 GAGCAGGAGA TTGACGGTGT GATGCGGATT TTTGGAGGCG TGCAGCAGCT CCGAGAGATC
TACAAGGACA ATCGGAACCT
321 GTTTGATGTG CAGGAGAATG AACCACAGAA ACTAGTGGAG AAGGTGGCAG GGGACATTGA
GAGCCTGCTG GACAGAAAGG
401 TCCAGGCCTT GAAGAGACTG GCTGACGCTG CAGAGAATTT CCAGAAAGCC CACCGCTGGC
AAGACAACAT CAAGGAGGAA
481 GACATCATGT ACTATGACGC CAAGGCTGAC GCCGAGCTGG ATGATCCTGA GAGTGAGGAT
ATGGAGAGGG GATCCAAGAC
561 CAGCGCCTTA AGGCTGGACT TCATCGAGGA GCCAACTTC AAGAACAAAG TCAACTATTC
ATACACGGCG GTGCAGATCC
641 CCACAGATAT CTACAAAGGC TCTACCGTCA TCCTCAATGA GCTTAACTGG ACGGAGGCCC
TGGAGAATGT CTTCAATTGAG
721 AACCGTAGGC AAGACCCTAC ACTGTTGTGG CAAGTCTTCG GCAGTGCCAC GGGAGTCACC
CGCTATTACC CAGCCACACC
801 GTGGCGAGCC CCAAGAAGA TTGACCTGTA CGATGTCAGA AGACGACCCT GGTATATACA
GGGGGCCTCA TCACCAAGG
881 ACATGGTCAT CATTGTGGAT GTGAGTGGA GTGTGAGCGG CCTGACGCTG AAGCTGATGA
AGACGTCTGT CTGTGAGATG
961 CTAGACACAC TCTCCGATGA TGACTATGTG AATGTGGCCT CATTCAACGA GAAGGCGCAG
CCTGTGTCTT GCTTCACACA
1041 CCTGGTGCAG GCCAATGTGC GGAACAAGAA GGTGTTCAAG GAAGCTGTGC AGGGCATGGT
GGCCAAGGGC ACCACAGGCT
1121 ACAAGGCTGG CTTTGAGTAT GCCTTTGACC AGCTACAGAA TTCCAACATC ACCCGTGCTA
ACTGCAATAA GATGATCATG
1201 ATGTTACCGG ACGGGGGAGA GGATCGCGTG CAGGACGTCT TTGAAAAGTA TAATTGGCCC
AATCGGACGG TACGCGTCTT
1281 CACGTTCTCC GTAGGACAGC ATAACATGA TGTCACACCC CTGCAGTGGA TGGCTTGTAC
TAACAAAGGT TACTATTTTG
1361 AGATCCCTTC CATCGGAGCC ATCCGCATCA ACACACAGGA ATACCTGGAT GTGCTGGGTA
GGCCCATGGT CCTGGCAGGC
1441 AAGGACGCCA AGCAAGTGCA ATGGACAAAC GTGTATGAAG ATGCGCTGGG GCTGGGGTTG
GTGGTAACAG GGAATCTCCC

```

1521 TGTTTTCAAC CTGACACAGG ATGGCCCTGG GGAAAAGAAG AACCAGCTAA TCCTGGGTGT
 CATGGGCATA GATGTGGCCC
 1601 TGAATGACAT CAAAAGGCTG ACTCCCAACT ACACACTTGG CGCCAATGGC TACGTGTTTCG
 CCATCGACCT GAATGGCTAT
 1681 GTGTTGCTAC ATCCCAATCT CAAGCCCCAG ACTACCAACT TCCGGGAGCC TGTGACCTTG
 GACTTCCTGG ATGCAGAGCT
 1761 GGAAGATGAG AACAAGGAGG AGATCCGTCG TAGTATGATT GACGAAGACA AAGGCCACAA
 GCAGATCAGA ACCTTGGTCA
 1841 AATCCCTGGA TGAGAGGTAC ATAGATGAAG TGATTTCGGAA CTACACCTGG GTGCCTATAA
 GGAGTACCAA CTACAGCCTG
 1921 GGGCTGGTGC TCCCACCCTA CAGCACCTAC TACCTCCAAG CCAACCTCAG CGACCAGATC
 CTGCAGGTCA AGTTGCCAAT
 2001 CAGCAAACTG AAGGATTTTG AGTTCCTGCT CCCCAGCAGC TTTGAGTCTG AAGGACATGT
 TTTTATTGCT CCCAGAGAGT
 2081 ATTGCAAGGA TTTGAATGCC TCAGACAACA ACACCGAGTT CCTGAAAAAC TTCATTGAGC
 TCATGGAGAA AGTGAATCCG
 2161 GACTCCAAGC AGTGCAATAA CTTCCCTTCTG CATAACTTGA TTTTGGACAC GGGCATTACG
 CAGCAGTTAG TGGAACGCGT
 2241 CTGGCGGGAC CAAGATCTCA ACACGTACAG CCTGCTAGCT GTATTTGCTG CCACTGACGG
 TGCAGTCACA CGTGTCTTCC
 2321 CGAACAAGGC AGCTGAAGAC TGGACAGAAA ACCCTGAACC CTTCAATGCC AGCTTCTACC
 GCCGCAGCCT GGATAACCGT
 2401 GGTTATATCT TCAAGCCCC GCACCAGGAC TCCCTGTTAA GGCCACTGGA GCTGGAGAAT
 GACACAGTAG GCGTCCTCGT
 2481 CAGCACAGCT GTAGAGCTCA GTCTGGGTCG CCGCACACTG AGGCCAGCAG TGGTGGGTGT
 CAAACTGGAC CTAGAGGCTT
 2561 GGGCTGAAAA GTTCAAGGTG CTTGCCAGCA ACCGTACCCA TCAGGACCAA CCTCAGAAGC
 AGTGCGGCCC CAGCAGCCAC
 2641 TGTGAGATGG ACTGCGAGGT AAACAACGAG GACCTACTCT GTGTCTCAT TGATGACGGG
 GGATTCTTGG TGCTGTCAA
 2721 CCAGAACCAC CAGTGGGACC AGGTTGGCAG ATTCTTCAGT GAGGTGGATG CCAACCTGAT
 GCTGGCACTG TACAATAACT
 2801 CCTTCTACAC CAGAAAGGAG TCCTATGACT ATCAGGCAGC TTGTGCCCCT CAGCCTCCTG
 GCAACCTGGG TGCTGCACCC
 2881 AGGGGTGTCT TTGTGCCAC CATTGCAGAT TTCCTTAACT TGGCCTGGTG GACCTCTGCT
 GCCGCCTGGT CCTTATTCCA
 2961 GCAACTACTT TATGGTCTCA TCTATCACAG CTGGTTCCAG GCAGACCCGG CAGAAGCCGA
 GGGCAGCCCC GAGACGCGCG
 3041 AGAGCAGCTG CGTCATGAAA CAAACCCAGT ACTACTTCGG CTCGGTGAAC GCGTCCTATA
 ACGCCATCAT CGACTGCGGA
 3121 AACTGCAGCA GGCTGTTCCA CGCGCAGAGA CTGACCAACA CCAACCTTCT GTTCGTGGTG
 GCGGAGAAGC CGCTGTGCAG
 3201 CCAGTGCGAG GTCGGCCGGC TGCTACAAAA GGAGACACAC TGCCCAGCGG ACGGCCCGGA
 GCAGTGTGAG CTGGTGCAGA
 3281 GACCGCGATA CCGAAGAGGC CCGCACATCT GTTTTACTA CAATGCGACG GAAGATACCT
 CAGACTGTGG CCGCGGCACG
 3361 TCCTTCCCTC CGTCGCTGGG CGTCTTGGTT TCCCTGCAGC TGTTGCTCCT CCTAGGCCTG
 CCACCTCGGC CGCAGCCTCA
 3441 AATCCATTCC TTCGCTGCCT CTCGCCGCCT CTGA

and the deduced amino acid sequence of rat brain $\alpha_2\delta$ -2 variant is SEQ ID NO:11.

[0072] Translated rat brain $\alpha_2\delta$ -2 variant amino acid (SEQ ID NO:11):

```

1  MAVPARTCGA SWPGPVRTAR PWPGRGPRPC PDPRGPASGP ARPLLLLLPP LLLLPLLTAP
   GASAYSFPQQ HTMQHWARRL
81  EQEIDGVMRI FGGVQQLREI YKDNRNLFDV QENEPQKLVE KVAGDIESLL DRKVQALKRL
   ADAAE NFQKA HRWQDNIKEE
161 DIMYYDAKAD AELDDPESED MERGSKTSAL RLD FIEEPNF KNKVNYSYTA VQIPTDIYKG
   STVILNELNW TEALENVFIE
241 NRRQDPTLLW QVFGSATGVT RYYPATPWRA PKKIDLYDVR RRPWYIQGAS SPKDMVIIVD
   VSGSVSGLTL KLMKTSVCEM
321 LDTLSDDDYV N VASFNEKAQ PVSCFTHLVQ ANVRNKKVKF EAVQGMVAKG TTGYKAGFEY
   AFDQLQNSNI TRANCNMIM
401 MFTDGGEDRV QDVFEKYNWP NRTVRVFTFS VGQHNYDVTP LQWMACTNKG YYFEIPSIGA
   IRINTQEYLD VLGRPMVLG
481 KDAKQVQWTN VYEDALGLGL VVTGTLPVFN LTQDGPGEKK NQLILGVMGI DVALNDIKRL
   TPNYTLGANG YVFAIDLNGY
561 VLLHPNLKPQ TTNFREPRTL DFLDAELEDE NKEEIRRSMI DEDKGHKQIR TLVKSLDERY
   IDEVIRNYTW VPIRSTNYSL
641 GLVLPPYSTY YLQANLSDQI LQVKLPISKL KDFEFLLPSS FESEGHVFIA PREYCKDLNA
   SDNNT EFLKN FIELMEKVTP
721 DSKQCNNFLL HNLILDTGIT QQLVERVWRD QDLNTYSLA VFAATDGAVT RVFPNKAED
   WTENPEPFNA SFYRRSLDNR
801 GYIFKPPHQD SLLRPLELEN DTVGVLVSTA VELSLGRRTL RPAVVGVKLD LEAWAEKFKV
   LASNRTHQDQ PQKQCGPSSH
881 CEMDCEVNNE DLLCVLIDDG GFLVLSNQNH QWDQVGRFFS EVDANLMLAL YNNSFYTRKE
   SYDYQAACAP QPPGNLGAAP
961 RGVFVPTIAD FLNLAWWTS AAWSLFQQLL YGLIYHSWFQ ADPAEAEAGSP ETRESSCVMK
   QTQYYFGSVN ASYNAIIDCG
1041 NCSRLFHAQR LTNTNLLFVV AEKPLCSQCE VGRLLQKETH CPADGPEQCE LVQRPRYRRG
   PHICFDYNAT EDTSDCGRGT
1121 SFPPSLGVLV SLQLLLLLGL PPRPQPQIHS FAASRRL*

```

Example 2**Heterologous Expression of Rat $\alpha_2\delta$ -2 Calcium Channel Subunits in Cells****A. Transient Transfection in Mammalian Cells**

[0073] Human embryonic kidney cells, HEK 293 (ATCC# CRL 1573) or HEK 293 tsA 201, were grown in standard DMEM medium supplemented with 4 mM glutamine and 10% fetal bovine serum. The rat $\alpha_2\delta$ -2 construct was transfected into HEK 293 or HEK 293 tsA 201 cells with equimolar concentration of the subunits α_{1B} (N-type calcium channel) and β_{1B} , using lipofectamine (Gibco/Invitrogen) according to the manufacturer's instructions. pEGFP was included in the transfected cDNA at 3-5 times less the molar concentration to detect transfected cells and to determine the efficiency of transfection. Transfected cells were incubated at 37°C, 5% CO₂, for 6-24 hours and then placed at 29°C, 5% CO₂.

[0074] After an incubation period of 24 to 72 hours, the culture medium was removed and replaced with an external recording solution containing (in mM) 5 BaCl, 129 CsCl, 1 MgCl₂, 10 HEPES, 10 glucose, pH 7.4 with CsOH. Whole-cell patch clamp recordings were made with an Axopatch 200B amplifier (Axon Instruments, USA). Recording electrodes with typical resistances of 4-8 MO were backfilled with (in mM) 108 caesium-methanesulfonate, 2 MgCl₂, 10 HEPES, 11 EGTA, 2 ATP, pH 7.2 with some CsOH. To create command potentials and acquire data, Clampex 8.2 software (Axon Instruments, USA) and a Digidata 1322A A/D converter interface (Axon Instruments, USA) were used. Currents were elicited at test potential of +20 mV (50 ms duration) from a holding potential of -80 mV. Leak and capacitance currents were subtracted on-line with a standard P/4 protocol. Evoked currents were filtered by a low-pass Bessel filter set at 1 kHz. Signals were acquired at 2.02 kHz and analyzed offline using pClamp 8.2 (Axon Instruments, USA) and Origin (OriginLab Corporation, USA) software. The effects of coexpressing the rat $\alpha_2\delta$ -2 auxiliary subunit of Example 1 with the rat $\alpha_{1B} + \beta_{1b}$ calcium channel subunits were tested by comparing average current density ($I_{\text{peak}}/\text{cell capacitance}$) with and without $\alpha_2\delta$ -2.

[0075] Mean current density was recorded for 4 cells transfected with rat $\alpha_{1B} + \beta_{1b}$ only and for 7 cells that were also cotransfected with rat brain $\alpha_2\delta$ -2 subunit. The rat $\alpha_{1B} + \beta_{1b}$ current density was increased by ~4 fold from -7.2 ± 2.3 pA/pF to -30.0 ± 4.1 pA/pF when coexpressed with the rat brain $\alpha_2\delta$ -2 subunit. $P = 0.01$, Student's unpaired t -test. Values are the mean $\pm$ S.E.M (Figure 4).

B. Transient Transfection in Xenopus Oocytes

[0076] Stage V and VI *Xenopus* oocytes are prepared as described by Dascal, *et al.*, *Science* (1986) 231:1147-1150. After enzymatic dissociation with collagenase, oocyte nuclei are microinjected with the rat $\alpha_2\delta$ -2 subunit cDNA expression vector construct (approximately 10 ng DNA per nucleus) using a Drummond nanoject apparatus, alone, or in combination with other rat, human or other mammalian or animal calcium channel subunit cDNA's, such as the α_1 and β_{1b} subunit cDNA. After incubation from 48 to 96 hours macroscopic currents are recorded using a standard two microelectrode voltage-clamp (Axoclamp 2A, Axon Instruments, Burlingame, CA) in a bathing medium containing (in mM): 40 Ba(OH)₂, 25 TEA-OH, 25 NaOH, 2 CsOH, 5 HEPES (pH titrated to 7.3 with methan-sulfonic acid). Pipettes of typical resistance ranging from 0.5 to 1.5 mO are filled with 2.8M CsCl, 0.2M CsOH, 10 mM HEPES, 10 mM BAPTA free acid. Endogenous Ca (and Ba)--activated Cl currents are suppressed by

systematically injecting 10-30 nl of a solution containing 100 mM BAPTA-free acid, 10 mM HEPES (pH titrated to 7.2 with CsOH) using a third pipette connected to a pneumatic injector. Leak currents and capacitive transients are subtracted using a standard P/5 procedure.

Example 3

Construction of Stable Cell Lines Expressing Rat $\alpha_2\delta$ -2 Calcium Channels

[0077] Mammalian cell lines stably expressing the rat brain $\alpha_2\delta$ -2 calcium channel were made by transfecting an expression vector containing the $\alpha_2\delta$ -2 calcium channel cDNA plus the rat $\beta 1b$ cDNA into HEK 293F cells, and selecting for cells resistant to Zeocin. Briefly, the full-length rat $\alpha_2\delta$ -2 subunit cDNA (clone 27) was excised from the pBK-RSV vector by digestion with Hind III followed by treatment with the Klenow fragment of DNA polymerase I to generate a blunt end and subsequent digestion with Kpn I. The mammalian expression vector pBud CE 4 rat $\beta 1b$ clone 3 was digested with Not I followed by Klenow treatment and digestion with Kpn I. The rat $\alpha_2\delta$ -2 fragment was ligated (blunt/sticky) into pBud CE4 rat $\beta 1b$ to give a final construct pBud rat $\alpha_2\delta$ -2 / rat $\beta 1b$ clone 3. Prior to making stable cell lines expressing the two subunits, the expression and activity of the subunits were tested by transient transfection and patch clamp analysis (see protocol Example 2). The pBud rat $\alpha_2\delta$ -2 / rat $\beta 1b$ construct was linearized with Pvu I and transfected into HEK 293F cells using lipofectamine. The transfected cells are incubated for 16 hours at 37°C, 5% CO₂, then placed in nonselective medium for an additional 24 hours. The cells were trypsinized and plated at low density in selective medium containing Zeocin at 250 µg/ml. After 14-16 days in selective medium, cells that were resistant to Zeocin grew as visible colonies which were harvested by the pipet technique. Cell colonies were expanded to make frozen stocks of cells and to determine the level of rat $\alpha_2\delta$ -2 subunit expression. Rat $\alpha_2\delta$ -2 subunit expression levels for the cell lines were determined by Northern blotting and reverse-transcriptase PCR. Detection of functional calcium channels containing the rat $\alpha_2\delta$ -2 subunit in stably transfected cells is examined electrophysiologically, such as by whole cell patch clamp or single channel analysis (see above). Other means of detecting functional calcium channels include the use of radiolabeled ⁴⁵Ca uptake, or fluorescence spectroscopy using calcium sensitive dyes such as FURA-2.

Example 4

[0078] Novel $\alpha_2\delta$ -3 subunits of the invention were identified using RT-PCR of rat brain total RNA and by screening a rat brain cDNA library. Screening of the cDNA library followed

standard methods and included infecting bacteria with recombinant lambda phage and immobilizing lambda DNA onto nylon filters (Hybond-N, Amersham Pharmacia Biotech).

[0079] A rat $\alpha_2\delta$ -3 cDNA probe of 470 bp (1789-2258) was amplified from rat brain total RNA using One-Step RT-PCR (Superscript, Invitrogen) with primers designed to a conserved region of the mouse and human $\alpha_2\delta$ -3 genes as follows:

1789F 5'-GTGTCCTTGGCAGATGAATGGTCCTAC-3' (SEQ ID NO:18);

2258R 5'-GATGTACTTGCTGTCACCACATTGCT-3' (SEQ ID NO:19).

[0080] The resulting rat $\alpha_2\delta$ -3 PCR product was ligated into pGEM-T easy (Promega) (clone 1) and sequenced. The rat $\alpha_2\delta$ -3 cDNA fragment was excised from the pGEM vector with Not I and radiolabeled with [$a^{32}P$] dCTP using the Multi-Prime DNA Labeling System (Amersham). Free $a^{32}P$ dCTP was removed by passage through a Centri Spin-20 column (Princeton Separations). Approximately 300,000 plaque forming units (pfus) were screened with the radiolabeled probe using moderate hybridization (55°C) in Church and Gilbert's solution and a final high stringency wash condition of 0.2 X SSC/0.1% SDS (60°C, 30 min). Bacteriophage that hybridized to the rat $\alpha_2\delta$ -3 radiolabeled probe were purified by sequential rounds of screening and the phagemid cDNA (pBluescript SK+) isolated by *in vivo* excision using the ExAssist helper phage with SOLAR Strain *E. coli* according to the manufacturer's instructions (Stratagene).

[0081] Four positive clones were purified by excision, sequenced and found to contain cDNA fragments of the rat $\alpha_2\delta$ -3 mRNA. Two different size cDNA fragments were obtained, two of 1,625 bp and two of 1,407 bp, of which one clone from each size was completely sequenced. Both clones were missing approximately 1,500 bp of 5' ORF. Clone 7-2-1-4 (1,625 bp) had more 5' region, but truncated approximately 70 bp before the stop codon. Clone 7-3-1-1 (1,407 bp) starts at 1,766 bp and includes the stop signal and a portion of the 3' UTR, but is missing approximately 426 bp (2,451-2,877) of coding sequence in the 3' region of rat $\alpha_2\delta$ -3 mRNA. Translation of clone 7-3-1-1 into the protein sequence revealed that this clone is a splice variant with a deletion of 142 amino acids (Figure 3). In order to obtain the 5' region of the rat $\alpha_2\delta$ -3 mRNA, an antisense primer:

2021R 5'-ATCGCTTCCAGTTGAGAGAGATGG-3' (SEQ ID NO:20)

was made to the rat brain $\alpha_2\delta$ -3 cDNA and a sense primer to the rat atrial $\alpha_2\delta$ -3 cDNA sequence (AF486278, SEQ. ID. NO:4):

1F 5'-ATGGCCGGGCGGGCTCGCTGTGCT-3' (SEQ ID NO:21)

and used in One-Step RT-PCR to amplify a 2,018 bp cDNA fragment.

[0082] The PCR products were cloned into pGEM-T easy and two clones were completely sequenced and identified to be rat $\alpha_2\text{d-3}$. These clones extended from the start codon (ATG) to position 2,018 bp. A full-length rat $\alpha_2\text{d-3}$ cDNA was constructed using the PCR 5' fragment (clone 1) and the two clones 7-2-1-4 and 7-3-1-1. Clone 1 was digested with Not I/Sac I and the lambda clone 7-2-1-4 digested with Sac I/Kpn I. These two cDNA fragments were three way ligated into pBK/RSV at the Not I/Kpn I sites. The full-length rat $\alpha_2\text{d-3}$ cDNA was constructed in the mammalian expression vector pBud CE4 (Not I/Kpn I) using a three way ligation of a Not I/Ban I digested fragment of pBK/RSV clone 1/7-2-1-4 (Ban I cuts at position 3,087 in the cDNA) and a Ban I/Kpn I digested fragment of clone 7-3-1-1.

[0083] The full-length rat brain $\alpha_2\text{d-3}$ cDNA clone (clone 1/7-2-1-4) has SEQ. ID. NO:5:
Full-length rat brain $\alpha_2\text{d-3}$ calcium channel subunit nucleotide sequence (SEQ. ID. NO:5)

```

1  ATG GCC GGG CCG GGC TCG CTG TGC TGC GCG TCC CGG GGG GCC TCG GCG CTC CTA
   GCC ACC GCG CTT
67  CTC TAC GCC GCG CTG GGG GAC GTG GTG CGC TCC GAG CAG CAG ATC CCG CTC TCC
   GTA GTG AAG CTC
133 TGG GCC TCC GCT TTT GGT GGG GAG ATA AAA TCC ATT GCT GCC AAG TAC TCG GGT
   TCC CAG CTT CTG
199 CAA AAG AAA TAC AAA GAG TAT GAG AAA GAC GTT GCC ATT GAA GAA ATC GAC GGT
   CTC CAA CTG GTG
265 AAA AAG CTG GCC AAG AAC ATG GAA GAG ATG TTT CAC AAG AAG TCC GAG GCA GTG
   CGG CGT CTC GTG
331 GAG GCT GCA GAG GAA GCA CAC CTG AAG CAT GAA TTT GAC GCC GAC TTG CAG TAT
   GAA TAC TTC AAT
397 GCC GTG CTG ATC AAC GAG AGA GAC AAA GAT GGG AAC TTT TTG GAA TTG GGA AAG
   GAG TTC ATC TTG
463 GCC CCC AAT GAC CAT TTT AAT AAT TTG CCT GTG AAC ATC AGT CTG AGT GAT GTC
   CAA GTG CCA ACG
529 AAC ATG TAC AAC AAA GAT CCT GCC ATA GTC AAT GGA GTG TAT TGG TCT GAA TCT
   CTA AAC AAA GTT
595 TTT GTA GAC AAC TTC GAT CGG GAC CCG TCT CTC ATA TGG CAG TAC TTT GGA AGT
   GCA AAG GGC TTT
661 TTC AGA CAG TAC CCA GGG ATT AAA TGG GAA CCA GAC GAG AAT GGA GTC ATT GCC
   TTT GAC TGC AGG
727 AAC AGG AAA TGG TAC ATC CAG GCA GCA ACT TCT CCA AAG GAT GTG GTC ATT TTG
   GTG GAC GTC AGC
793 GGG AGC ATG AAA GGA CTC CGC CTG ACC ATC GCC AAG CAA ACA GTC TCC TCG ATA
   CTG GAC ACC CTG
859 GGC GAT GAT GAC TTC TTC AAC ATC ATC ACG TAT AAC GAA GAG CTT CAC TAT GTG
   GAA CCT TGT CTG
925 AAT GGA ACA CTG GTT CAA GCG GAC AGG ACC AAC AAG GAG CAC TTC AGG GAG CAT
   TTG GAC AAA CTT
991 TTT GCC AAA GGG ATT GGA ATG CTC GAT ATT GCG CTG AAC GAG GCC TTC AAT GTA
   CTG AGC GAT TTC
1057 AAC CAC ACC GGA CAA GGA AGC ATT TGC AGC CAG GCC ATT ATG CTC ATA ACC GAT
   GGG GCA GTG GAC

```

1123 ACC TAC GAC ACC ATC TTT GCA AAG TAC AAT TGG CCA GAG CGA AAG GTT CGA ATC
 TTC ACT TAC CTC
 1189 ATT GGA CGA GAG GCT GCT TTT GCA GAC AAT CTC AAG TGG ATG GCT TGT GCT AAC
 AAA GGA TTT TTC
 1255 ACC CAG ATC TCC ACC TTG GCT GAT GTG CAG GAA AAT GTC ATG GAA TAC CTC CAT
 GTA CTC AGT CGA
 1321 CCC AAA GTC ATC GAC CAG GAA CAT GAT GTG GTG TGG ACC GAA GCG TAC ATC GAC
 AGC ACT CTC CCT
 1387 CAG GCT CAA AAG CTT GCT GAT GAT CAG GGC CTC GTC TTG ATG ACC ACA GTG GCC
 ATG CCT GTG TTT
 1453 AGT AAG CAG AAC GAA ACT AGG TCA AAG GGC ATT CTT CTG GGT GTG GTT GGC ACA
 GAT GTC CCA GTA
 1519 AAA GAG CTT CTG AAG ACC ATC CCC AAA TAC AAG TTA GGA ATT CAT GGT TAT GCC
 TTT GCC ATC ACG
 1585 AAT AAT GGA TAC ATC TTG ACA CAC CCG GAG CTC AGG CCC CTG TAT GAA GAA GGG
 AAA AAG CGA AGG
 1651 AAG CCT AAT TAC AGT AGT GTG GAT CTC TCG GAA GTC GAG TGG GAA GAT CGG GAT
 GAT GTG TTA CGA
 1717 AAT GCC ATG GTG AAT CGG AAG ACT GGG AAA TTC TCC ATG GAA GTG AAG AAG ACT
 GTG GAC AAA GGG
 1783 AAA CGG GTT TTG GTG ATG ACC AAT GAC TAC TAC TAC ACA GAC ATC AAG GGT GCT
 CCT TTC AGT TTA
 1849 GGT GTG GCG CTC TCC AGG GGC CAC GGG AAA TAC TTC TTC CGA GGG AAT GTA ACC
 ATT GAA GAA GGG
 1915 CTC CAT GAC TTA GAA CAT CCT GAC GTG TCC TTG GCA GAT GAA TGG TCC TAC TGC
 AAC ACT GAT CTG
 1981 CAC CCA GAG CAC CGC CAT CTC TCT CAA CTG GAA GCG ATT AAG CTC TAC CTC AAA
 GGC AAG GAG CCT
 2047 CTG CTT CAA TGT GAC AAA GAA TTG ATT CAA GAA GTC CTT TTT GAT GCT GTG GTA
 AGC GCC CCT ATC
 2113 GAA GCC TAT TGG ACC AGC CTG GCC CTC AAC AAA TCT GAG AAT TCT GAC AAG GGT
 GTA GAG GTC GCC
 2179 TTC CTC GGC ACT CGC ACA GGC CTC TCA AGA ATC AAC CTG TTT GTG GGG GCT GAA
 CAG CTC ACC AAT
 2245 CAG GAC TTT CTG AAG GCT AGA GAC AAA GAG AAC ATT TTC AAC GCA GAT CAT TTC
 CCT CTC TGG TAC
 2311 AGA AGA GCT GCC GAG CAG ATT CCA GGA AGT TTT GTC TAC TCC ATC CCC TTC AGC
 ACA GGA ACG GTC
 2377 AAC AAA AGC AAT GTG GTG ACA GCA AGT ACC TCC ATC CAA CTC CTG GAT GAG CGA
 AAA TCT CCT GTG
 2443 GTG GCA GCT GTA GGC ATT CAG ATG AAA CTT GAA TTC TTC CAA AGG AAG TTC TGG
 ATG GCC AGC AGA
 2509 CAG TGT GCC TCC CTG GAT GGT AAA TGC TCC ATA AGC TGC GAC GAT GAG ACT GTG
 AAC TGT TAC CTC
 2575 ATA GAC AAT AAC GGG TTC ATT CTG GTG TCT GAA GAC TAC ACA CAG ACT GGA GAT
 TTT TTT GGT GAG
 2641 GTC GAA GGA GCT GTC ATG AAC AAG TTG TTA ACA ATG GGC TCC TTT AAA AGA ATA
 ACC TTG TAT GAC
 2707 TAC CAA GCC ATG TGT AGA GCC AAC AAG GAG AGT AGT GAC AGT GCC CAC GGA CTC
 CTG GAC CCC TAT
 2773 AAG GCC TTC CTC TCT GCA GCC AAG TGG ATA GTG ACG GAA CTT GTC TTG TTC CTG
 GTG GAG TTT AAC

2839 CTT TGC AGT TGG TGG CAC TCT GAC ATG ACA GCT AAA GCC CAG AAA CTG AAA CAG
 ACC CTG GAG CCT
 2905 TGT GAT ACT GAA TAC CCA GCC TTT GTT TCC GAA CGC ACC ATC AAG GAG ACC ACA
 GGG AAC ATT GCT
 2971 TGT GAA GAC TGC TCC AAG TCC TTT GTC ATC CAG CAA ATC CCA AGT AGC AAT CTG
 TTC ATG GTG GTG
 3037 GTG GAC AGT AGC TGT CTC TGT GAG TCT GTG GCT CCT ATC ACC ATG GCA CCC ATT
 GAA ATC AGG TAT
 3103 AAT GAA TCC CTT AAG TGT GAA CGG TTA AAG GCT CAG AAG ATC AGA CGA CGT CCG
 GAA TCC TGC CAC
 3169 GGC TTC CAT CCT GAG GAG AAT GCG AGA GAG TGT GGG GGT GCA TCA AGT CTC CAG
 GCC CAG GTG GCC
 3235 TTG CTG CTG CTC CCC CTG GTT TCG AGT CTC TTC TCA AGG **TGA** CAC TAA CTA ATG
 GGA TGT TCT TTT
 3301 GGC ATG CTA TAA ATC ATG GAT AAA CTG TGA ACC CAA CTA TGG TGC GAC ATA GAA
 GAC ATA AGC ATA
 3367 GCC CAG CCA TCA GCA TCT CAT GAT TTT AAA CTG TGT GTG ATA GAA ACT CTA ACA
 GGT ACA CTG ACC
 3433 AAA AGT TCT CTT TTT ACT TTG CCA ATC ATG CAA ATG TGA GTG CCA CAT GAC CAC
 CCT TCA TCA GAA
 3499 ATG GGG CTG TAC TGG GTA GGC AGT GGC CTT CTG CTT GAA AAC CAT GGA AAC CAA
 TTT AAA ACT GTG
 3565 TAC TTT TTA AAT AAA GTA TAT TAA AAT CAT AAA A

and the deduced amino acid sequence of full-length rat brain α_2 -3 subunit is SEQ. ID. NO:6.

[0084] Translated full-length rat brain α_2 -3 amino acid sequence (SEQ. ID. NO:6)

1 MAGPGSLCCA SRGASALLAT ALLYAALGDV VRSEQQIPLS VVKLWASAFG
 51 GEIKSIAAKY SGSQLLQKKY KEYEKDVAIE EIDGLQLVKK LAKNMEEMFH
 101 KKSEAVRRLV EAAEEAHLKH EFDADLQY EY FNAVLINERD KDGNFLELGK
 151 EFILAPNDHF NNLFPVNISLS DVQVPTNMYN KDPAIVNGVY WSESLNKVVFV
 201 DNFDRDPSLI WQYFGSAKGF FRQYPGIKWE PDENGVI AFD CRNRKWIYQA
 251 ATSPKDVVIL VDVSGSMKGL RLTI AKQTVS SILD TLGDDD FFNIITYNEE
 301 LHYVEPCLNG TLVQADRTNK EHFREHLDKL FAKGIGMLDI ALNEAFNVLS
 351 DFNHTGQGS I CSQA IMLITD GAVDTYDTIF AKYNWPERKV RIFTY LIGRE
 401 AAFADNLKWM ACANKGFFTQ ISTLADVQEN VMEYLHVLSR PKVIDQEHDV
 451 VWTEAYIDST LPQAQKLADD QGLVLMTTVA MPVFSKQNET RSKGILLGVV
 501 GTDVPVKELL KTIPKYKLG I HGYAFAITNN GYILTHPELR PLYEEGKKRR
 551 KPNYSSVDLS EVEWEDRDDV LRNAMVNRKT GKFSMEVKKT VDKGKRVLMV
 601 TNDYYYTDIK GAPFSLGVAL SRGHGKYFFR GNV TIEEGLH DLEHPDVSLA
 651 DEWSYCN TDL HPEHRHLSQL EAIKLYLK GK EPLLQCDKEL IQEVLFD AVV
 701 SAPIEAYWTS LALNKSENSD KGVEVAFLGT RTGLSRINLF VGAEQLTNQD
 751 FLKARDKENI FNADHFPLWY RRAAEQIPGS FVYSIPFSTG TVNKS NVVTA
 801 STSIQLLDER KSPVVA AVGI QMKLEFFQRK FWMASRQCAS LDGKCSISCD
 851 DETVNCYLID NNGFILVSED YTQTG DFFGE VEGAVMNKLL TMGSFKRITL
 901 YDYQAMCRAN KESSDSAHGL LDPYKAFLSA AKWIVTELVL FLVEFNLC SW
 951 WHSDMTAKAQ KLKQ TLEPCD TEYPAFV SER TIKETTGNIA CEDCSKSFVI
 1001 QQIPSSNLFM VVVDSSCLCE SVAPITMAPI EIRYNESLKC ERLKAQKIRR
 1051 RPESCHGFHP EENARECGGA SSLQAQVALL LLPLVSSLFS R*

[0085] The rat brain $\alpha_2\delta$ -3 truncated variant cDNA clone (clone 1/7-3-1-1) has SEQ.

ID. NO:8: Truncated rat brain $\alpha_2\delta$ -3 variant calcium channel subunit nucleotide sequence
(SEQ ID NO:8)

```

1   ATGGCCGGGC CGGGCTCGCT GTGCTGCGCG TCCCGGGGGG CCTCGGCGCT
51  CCTAGCCACC GCGCTTCTCT ACGCCGCGCT GGGGGACGTG GTGCGCTCCG
101 AGCAGCAGAT CCCGCTCTCC GTAGTGAAGC TCTGGGCCTC CGCTTTTGGT
151 GGGGAGATAA AATCCATTGC TGCCAAGTAC TCGGGTTCCC AGCTTCTGCA
201 AAAGAAATAC AAAGAGTATG AGAAAGACGT TGCCATTGAA GAAATCGACG
251 GTCTCCAAC TGTGAAAAAG CTGGCCAAGA ACATGGAAGA GATGTTTCAC
301 AAGAAGTCCG AGGCAGTGCG GCGTCTCGTG GAGGCTGCAG AGGAAGCACA
351 CCTGAAGCAT GAATTTGACG CCGACTTGCA GTATGAATAC TTCAATGCCG
401 TGCTGATCAA CGAGAGAGAC AAAGATGGGA ACTTTTGGGA ATTGGGAAAG
451 GAGTTCATCT TGGCCCCCAA TGACCATTTT AATAATTTGC CTGTGAACAT
501 CAGTCTGAGT GATGTCCAAG TGCCAACGAA CATGTACAAC AAAGATCCTG
551 CCATAGTCAA TGGAGTGTAT TGGTCTGAAT CTCTAAACAA AGTTTTTGTA
601 GACAACCTCG ATCGGGACCC GTCTCTCATA TGGCAGTACT TTGGAAGTGC
651 AAAGGGCTTT TTCAGACAGT ACCCAGGGAT TAAATGGGAA CCAGACGAGA
701 ATGGAGTCAT TGCCTTTGAC TGCAGGAACA GGAAATGGTA CATCCAGGCA
751 GCAACTTCTC CAAAGGATGT GGTCAATTTG GTGGACGTCA GCGGGAGCAT
801 GAAAGGACTC CGCTGACCA TCGCCAAGCA AACAGTCTCC TCGATACTGG
851 ACACCCTGGG CGATGATGAC TTCTTCAACA TCATCACGTA TAACGAAGAG
901 CTTCACTATG TGGAACCTTG TCTGAATGGA ACACTGGTTC AAGCGGACAG
951 GACCAACAAG GAGCACTTCA GGGAGCATT TGGACAACTT TTTGCCAAAG
1001 GGATTGGAAT GCTCGATATT GCGCTGAACG AGGCCTTCAA TGTA CTGAGC
1051 GATTTCAACC ACACCGGACA AGGAAGCATT TGCAGCCAGG CCATTATGCT
1101 CATAACCGAT GGGGCAGTGG ACACCTACGA CACCATCTTT GCAAAGTACA
1151 ATTGGCCAGA GCGAAAGGTT CGAATCTTCA CTTACCTCAT TGGACGAGAG
1201 GCTGCTTTTG CAGACAATCT CAAGTGGATG GCTTGTGCTA ACAAAGGATT
1251 TTTCAACCCG ATCTCCACCT TGGCTGATGT GCAGGAAAAT GTCATGGAAT
1301 ACCTCCATGT ACTCAGTCGA CCCAAAGTCA TCGACCAGGA ACATGATGTG
1351 GTGTGGACCG AAGCGTACAT CGACAGCACT CTCCCTCAGG CTCAAAAGCT
1401 TGCTGATGAT CAGGGCCTCG TCTTGATGAC CACAGTGGCC ATGCCTGTGT
1451 TTAGTAAGCA GAACGAAACT AGGTCAAAGG GCATTCTTCT GGGTGTGGTT
1501 GGCACAGATG TCCCAGTAAA AGAGCTTCTG AAGACCATCC CCAAATACAA
1551 GTTAGGAATT CATGGTTATG CCTTTGCCAT CACGAATAAT GGATACATCT
1601 TGACACACCC GGAGCTCAGG CCCCTGTATG AAGAAGGGAA AAAGCGAAGG
1651 AAGCCTAATT ACAGTAGTGT GGATCTCTCG GAAGTCGAGT GGGAAGATCG
1701 GGATGATGTG TTACGAAATG CCATGGTGAA TCGGAAGACT GGGAAATTCT
1751 CCATGGAAGT GAAGAAGACT GTGGACAAAG GGAACGGGT TTTGGTGATG
1801 ACCAATGACT ACTACTACAC AGACATCAAG GGTGCTCCTT TCAGTTTAGG
1851 TGTGGCGCTC TCCAGGGGCC ACGGGAAATA CTTCTTCCGA GGGAAATGTAA
1901 CCATTGAAGA AGGGCTCCAT GACTTAGAAC ATCCTGACGT GTCCTTGGA
1951 GATGAATGGT CCTACTGCAA CACTGATCTG CACCCAGAGC ACCGCCATCT
2001 CTCTCAACTG GAAGCGATTA AGCTCTACCT CAAAGGCAAG GAGCCTCTGC
2051 TTCAATGTGA CAAAGAATTG ATTCAAGAAG TCCTTTTTGA TGCTGTGGTA
2101 AGCGCCCTTA TCGAAGCCTA TTGGACCAGC CTGGCCCTCA ACAAATCTGA
2151 GAATTCTGAC AAGGGTGTAG AGGTGCGCTT CCTCGGCACT CGCACAGGCC
2201 TCTCAAGAAT CAACCTGTTT GTGGGGGCTG AACAGCTCAC CAATCAGGAC
2251 TTTCTGAAGG CTAGAGACAA AGAGAACATT TTCAACGCAG ATCATTTCCT
2301 TCTCTGGTAC AGAAGAGCTG CCGAGCAGAT TCCAGGAAGT TTTGTCTACT
2351 CCATCCCCTT CAGCACAGGA ACGGTCAACA AAAGCAATGT GGTGACAGCA
2401 AGTACCTCCA TCCAACCTCT GGATGAGCGA AAATCTCCTG TGGTGGCAGC
2451 CCAGAACTG AAACAGACCC TGGAGCCTTG TGATACTGAA TACCCAGCCT
2501 TTGTTTCCGA ACGCACCATC AAGGAGACCA CAGGGAACAT TGCTTGTGAA
2551 GACTGCTCCA AGTCCTTTGT CATCCAGCAA ATCCCAAGTA GCAATCTGTT
2601 CATGGTGGTG GTGGACAGTA GCTGTCTCTG TGAGTCTGTG GCTCCTATCA

```

```

2651 CCATGGCACC CATTGAAATC AGGTATAATG AATCCCTTAA GTGTGAACGG
2701 TTAAAGGCTC AGAAGATCAG ACGACGTCCG GAATCCTGCC ACGGCTTCCA
2751 TCCTGAGGAG AATGCGAGAG AGTGTGGGGG TGCATCAAGT CTCCAGGCCC
2801 AGGTGGCCTT GCTGCTGCTC CCCCTGGTTT CGAGTCTCTT CTCAAGGTGA
2851 CACTAACTAA TGGGATGTTT TTTTGGCATG CTATAAATCA TGGATAAACT
2901 GTGAACCCAA CTATGGTGCG ACATAGAAGA CATAAGCATA GCCCAGCCAT
2951 CAGCATCTCA TGATTTTAAA CTGTGTGTGA TAGAACTCT AACAGGTACA
3001 CTGACCAAAA GTTCTCTTTT TACTTTGCCA ATCATGCAAA TGTGAGTGCC
3051 ACATGACCAC CCTTCATCAG AAATGGGGCT GTACTGGGTA GGCAGTGGCC
3101 TTCTGCTTGA AAACCATGGA AACCAATTTA AAAGTGTGTA CTTTTTAAAT
3151 AAAGTATATT AAAATCATAA AA

```

and the deduced amino acid sequence of rat brain α_2 -3 truncated variant is SEQ. ID. NO:9:

[0086] Translated rat brain α_2 -3 variant amino acid sequence (SEQ. ID. NO:9)

```

1  MAGPGSLCCA SRGASALLAT ALLYAALGDV VRSEQQIPLS VVKLWASAFG
51  GEIKSIAAKY SGSQLLQKKY KEYEKDVAIE EIDGLQLVKK LAKNMEEMFH
101 KKSEAVRRLV EAAEEAHLKH EFDADLQY EY FNAVLINERD KDNFLELGK
151 EFILAPNDHF NNLPVNISLS DVQVPTNMYN KDPAIVNGVY WSESLNKV FV
201 DNFDRDPSLI WQYFGSAKGF FRQYPGIKWE PDENGVI AFD CRNRKWIQA
251 ATSPKDVVIL VDVSGSMKGL RLTI AKQTVS SILD TLGDDD FFNIITYNEE
301 LHYVEPCLNG TLVQADRTNK EHFREHL DKL FAKGIGMLDI ALNEAFNVLS
351 DFNHTQGQSI CSQAIMLITD GAVDTYD TIF AKYNWPERKV RIFTY LIGRE
401 AAFADNLKWM ACANKGFFTQ ISTLADVQEN VMEYLHVLSR PKVIDQEH DV
451 VWTEAYIDST LPQAQKLADD QGLVLMTTVA MPVFSKQNET RSKGILLGVV
501 GTDVPVKELL KTIPKYKLG I HG YAFAITNN GYILTHPELR PLYEEGKKRR
551 KPNYSSVDLS EVEWEDRDDV LRNAMVNRKT GKFSMEVKKT VDKGKRV LVM
601 TNDYYYTDIK GAPFSLGVAL SRGHGKYFFR GNV TIEEGLH DLEHPDVSLA
651 DEWSYCN TDL HPEHRHLSQL EAIKLYLK GK EPLLQCDKEL IQEVLFD AVV
701 SAPIEAYWTS LALNKSENSD KGVEVAFLGT RTGLSRINLF VGAEQLTNQD
751 FLKARDKENI FNADHFPLWY RRAAEQIPGS FVYSIPFSTG TVNKSNNV VTA
801 STSIQLLDER KSPVVAQKL KQTL EPCDTE YPAFVSERTI KETTGN IACE
851 DCSKSFVIQQ IPSSNLFMVV VDSSCLCESV APITMAPIEI RYNESLKCER
901 LKAQKIRRRP ESCHGFHPEE NARECGGASS LQAQVALLLL PLVSSLSFSR*

```

Example 5:

Screening for Calcium Channel Blockers

[0087] The whole-cell patch clamp recording technique used is as described in Example 2 to screen for calcium channel blockers. Test compounds are added daily to the extracellular recording solution from a stock concentration of 1 mM (in DMSO) for each experiment. Compounds are then applied directly onto the HEK cells by means of a custom-made gravity-driven perfusion system, which changes the solution around the cell in less than 1 s. Differences in the mean peak current densities in the absence and presence of compounds are measured to estimate the concentration of compound required to occupy 50 % of the channels (K_d). The K_d is estimated using the equation $K_d = a[\text{drug}]/1-a$.

Claims

1. An isolated nucleic acid comprising a nucleotide sequence that encodes a calcium channel subunit having the amino acid sequence of SEQ. ID. NO:3, 6, 9 or 11, or a fragment thereof which encodes a functional α_2 -2 or a functional α_2 -3 subunit.
2. A recombinant nucleic acid molecule which comprises a nucleotide sequence as set forth in claim 1, operably linked to control sequences to effect its expression.
3. The nucleic acid of claim 2, wherein said control sequences are operable in vertebrate cells.
4. Recombinant host cells which contain the nucleic acid molecule of claim 2.
5. Vertebrate host cells which contain the nucleic acid molecule of claim 3.
6. The vertebrate host cells of claim 5, wherein said cells further comprise an expression vector which comprises an expression control sequence operably linked to a nucleotide sequence which encodes an α_1 calcium channel subunit.
7. An isolated protein that functions as a calcium channel subunit which protein comprises the amino acid sequence set forth in SEQ. ID. NO:3, 6, 9 or 11, or a functional fragment thereof.
8. A method to prepare cells that display functional calcium ion channel, which method comprises culturing cells which display an α_1 subunit and which further comprise the nucleic acid molecule of claim 2.
9. Recombinant cells prepared by the method of claim 8.

10. A method to identify a compound that is an agonist or antagonist of calcium ion channel activity, which method comprises culturing the cells of claim 9 in the presence and in the absence of a candidate compound,

whereby an increase in calcium ion flow in the presence as opposed to the absence of said compound identifies said candidate compound as an agonist; and

whereby a decrease in the calcium ion flow in the presence as opposed to the absence of said compound identifies said candidate compound as an antagonist.

11. A nucleic acid probe which comprises at least 12 consecutive nucleotides of SEQ. ID. NO:2 from about nucleotide number 2,010 to about nucleotide number 2,027, or the complement thereof.

12. A method to detect the production of mRNA encoding a protein according to SEQ. ID. NO:3, which method comprises contacting mRNA to be assessed for the presence of said SEQ. ID. NO:3 protein encoding RNA with the probe of claim 11 under conditions of high stringency whereby hybridization of said probe to said RNA under said conditions demonstrates the production of SEQ. ID. NO:3 protein encoding RNA in said sample.

13. A nucleic acid probe which comprises at least 12 consecutive nucleotides of SEQ. ID. NO:5 from about nucleotide number 1,381 to about nucleotide number 1,398, or the complement thereof.

14. A method to detect the production of mRNA encoding a protein according to SEQ. ID. NO:6 or 9, which method comprises contacting mRNA to be assessed for the presence of said SEQ. ID. NO:6 or 9 protein encoding RNA with the probe of claim 13 under conditions of high stringency whereby hybridization of said probe to said RNA under said conditions demonstrates the production of SEQ. ID. NO:6 or 9 protein encoding RNA in said sample.

15. A nucleic acid probe which comprises at least 12 consecutive nucleotides of SEQ. ID. NO:8 from about nucleotide number 2,439 to about nucleotide number 2,458, or the complement thereof.

16. A method to detect the production of mRNA encoding a protein according to SEQ. ID. NO:9, which method comprises contacting mRNA to be assessed for the presence of said SEQ. ID. NO:9 protein encoding RNA with the probe of claim 15 under conditions of high stringency whereby hybridization of said probe to said RNA under said conditions demonstrates the production of SEQ. ID. NO:9 protein encoding RNA in said sample.

17. Antibodies or immunospecific fragments thereof that are immunospecific for the protein of claim 7, wherein the protein is a $\alpha_2\delta$ -2 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:3, or a functional fragment thereof.

18. A method to detect the presence of a $\alpha_2\delta$ -2 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:3 or a functional fragment thereof, which method comprises contacting a sample suspected of containing said protein with said antibodies or said immunospecific fragments of claim 17 under conditions whereby a complex between said antibodies and any $\alpha_2\delta$ -2 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:3, or a functional fragment thereof, present is formed and detecting the presence, absence or amount of said complex.

19. Antibodies or immunospecific fragments thereof that are immunospecific for the protein of claim 7, wherein the protein is a $\alpha_2\delta$ -3 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:6, or a functional fragment thereof.

20. A method to detect the presence of a $\alpha_2\delta$ -3 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:6 or a functional fragment thereof, which method comprises contacting a sample suspected of containing said protein with said antibodies or said immunospecific fragments of claim 19 under conditions whereby a complex between said antibodies and any $\alpha_2\delta$ -3 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:6, or a functional fragment thereof, present is formed and detecting the presence, absence or amount of said complex.

21. Antibodies or immunospecific fragments thereof that are immunospecific for the protein of claim 7, wherein the protein is a $\alpha_2\delta$ -3 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:9, or a functional fragment thereof.

22. A method to detect the presence of a $\alpha_2\delta$ -3 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:9 or a functional fragment thereof, which method comprises contacting a sample suspected of containing said protein with said antibodies or said immunospecific fragments of claim 21 under conditions whereby a complex between said antibodies and any $\alpha_2\delta$ -3 protein which comprises the amino acid sequence set forth in SEQ. ID. NO:9, or a functional fragment thereof, present is formed and detecting the presence, absence or amount of said complex.

23. A method for mapping the distribution of calcium channel subunit expression within a tissue sample comprising the steps of exposing the tissue to a reagent comprising a directly or indirectly detectable label coupled to a nucleic acid sequence comprising a sequence selected from among those sequences of SEQ. ID. NO:2, 5, 8 or 10, and detecting reagent that has bound to the tissue.

Rat brain a2d-2 (SEQ ID NO 3) vs rat atria a2d-2 (AF486277)

		1	50
AF486277	(1)	MAVPARTCGASWPGPVRTARPWPGRGPRPCPDPRGPASGPAPRLLLLP	
SEQ ID No 3	(1)	MAVPARTCGASWPGPVRTARPWPGRGPRPCPDPRGPASGPAPRLLLLP	
Consensus	(1)	MAVPARTCGASWPGPVRTARPWPGRGPRPCPDPRGPASGPAPRLLLLP	
		51	100
AF486277	(51)	LLLLPLLTAPGASAYSFPQOHTMOHWARRLEQEI DGVMRI FGQVQQLREI	
SEQ ID No 3	(51)	LLLLPLLTAPGASAYSFPQOHTMOHWARRLEQEI DGVMRI FGQVQQLREI	
Consensus	(51)	LLLLPLLTAPGASAYSFPQOHTMOHWARRLEQEI DGVMRI FGQVQQLREI	
		101	150
AF486277	(101)	YKDNRNLFQVQENEPQKLVKQVAGDIESLLDRKVQALKRLADAAENFQKA	
SEQ ID No 3	(101)	YKDNRNLFQVQENEPQKLVKQVAGDIESLLDRKVQALKRLADAAENFQKA	
Consensus	(101)	YKDNRNLFQVQENEPQKLVKQVAGDIESLLDRKVQALKRLADAAENFQKA	
		151	200
AF486277	(151)	HRWQDNKEEDIMYYDAKADAE LDDPESEDMERGSKTSALRLDFIEEPNF	
SEQ ID No 3	(151)	HRWQDNKEEDIMYYDAKADAE LDDPESEDMERGSKTSALRLDFIEEPNF	
Consensus	(151)	HRWQDNKEEDIMYYDAKADAE LDDPESEDMERGSKTSALRLDFIEEPNF	
		201	250
AF486277	(201)	KNKVNYSYTA VQIPTDIYKGSTVILNELNWEALENVFIENRRQDPTLLW	
SEQ ID No 3	(201)	KNKVNYSYTA VQIPTDIYKGSTVILNELNWEALENVFIENRRQDPTLLW	
Consensus	(201)	KNKVNYSYTA VQIPTDIYKGSTVILNELNWEALENVFIENRRQDPTLLW	
		251	300
AF486277	(251)	QVFGSATGVTRYYPATPWAPKKIDLYDVRPPWYIQGASSPKDMVIIVD	
SEQ ID No 3	(251)	QVFGSATGVTRYYPATPWAPKKIDLYDVRPPWYIQGASSPKDMVIIVD	
Consensus	(251)	QVFGSATGVTRYYPATPWAPKKIDLYDVRPPWYIQGASSPKDMVIIVD	
		301	350
AF486277	(301)	VSGSVSGLTLKLMKTSVCEMLDLSDDDYVNVASFNEKAQPVSCFTHLVQ	
SEQ ID No 3	(301)	VSGSVSGLTLKLMKTSVCEMLDLSDDDYVNVASFNEKAQPVSCFTHLVQ	
Consensus	(301)	VSGSVSGLTLKLMKTSVCEMLDLSDDDYVNVASFNEKAQPVSCFTHLVQ	
		351	400
AF486277	(351)	ANVRNKKVFKEAVQGMVAKGTTGYKAGFEYAFDQLQNSNITRANCNMIM	
SEQ ID No 3	(351)	ANVRNKKVFKEAVQGMVAKGTTGYKAGFEYAFDQLQNSNITRANCNMIM	
Consensus	(351)	ANVRNKKVFKEAVQGMVAKGTTGYKAGFEYAFDQLQNSNITRANCNMIM	
		401	450
AF486277	(401)	MFTDGGEDRVQDVFEKYNWPNRTVRVFTFSVGQHNVDVTPLOWMACNKG	
SEQ ID No 3	(401)	MFTDGGEDRVQDVFEKYNWPNRTVRVFTFSVGQHNVDVTPLOWMACNKG	
Consensus	(401)	MFTDGGEDRVQDVFEKYNWPNRTVRVFTFSVGQHNVDVTPLOWMACNKG	
		451	500
AF486277	(451)	YYFEIPSIGAIRINTQEYLDVLGRPMVLGKDAKQVQWNTNVYEDALGLGL	
SEQ ID No 3	(451)	YYFEIPSIGAIRINTQEYLDVLGRPMVLGKDAKQVQWNTNVYEDALGLGL	
Consensus	(451)	YYFEIPSIGAIRINTQEYLDVLGRPMVLGKDAKQVQWNTNVYEDALGLGL	
		501	550
AF486277	(501)	VVTGTLPVFNLTQDGPGEKKKNQLILGVMGIDVALNDIKRLTPNYTLGANG	
SEQ ID No 3	(501)	VVTGTLPVFNLTQDGPGEKKKNQLILGVMGIDVALNDIKRLTPNYTLGANG	
Consensus	(501)	VVTGTLPVFNLTQDGPGEKKKNQLILGVMGIDVALNDIKRLTPNYTLGANG	
		551	600
AF486277	(551)	YVFAIDLNGYVLLHPNLKPQITNFREPVTLDFLDAELEDENKEEIRRSMI	
SEQ ID No 3	(551)	YVFAIDLNGYVLLHPNLKPQITNFREPVTLDFLDAELEDENKEEIRRSMI	
Consensus	(551)	YVFAIDLNGYVLLHPNLKPQITNFREPVTLDFLDAELEDENKEEIRRSMI	
		601	650
AF486277	(601)	DGDKGHKQIRTLVKSLDERYIDEVIRNYTWVPIRSTNYSGLVLPYSTY	
SEQ ID No 3	(601)	DEDKGHKQIRTLVKSLDERYIDEVIRNYTWVPIRSTNYSGLVLPYSTY	
Consensus	(601)	D DKGHKQIRTLVKSLDERYIDEVIRNYTWVPIRSTNYSGLVLPYSTY	
		651	700
AF486277	(651)	YLQANLSDQILQVKLPISKLKDFEFLPSSFESEGHVFIAPREYCKDLNA	
SEQ ID No 3	(651)	YLQANLSDQILQVKY-----FEFLPSSFESEGHVFIAPREYCKDLNA	
Consensus	(651)	YLQANLSDQILQVK FEFLPSSFESEGHVFIAPREYCKDLNA	
		701	750
AF486277	(701)	SDNNTFLKNFIELMEKVTPDSKQCNNFLLHNLILDTGITQQLVERVWRD	
SEQ ID No 3	(694)	SDNNTFLKNFIELMEKVTPDSKQCNNFLLHNLILDTGITQQLVERVWRD	
Consensus	(701)	SDNNTFLKNFIELMEKVTPDSKQCNNFLLHNLILDTGITQQLVERVWRD	

Figure 1A

		751	800
AF486277	(751)	QDLNTYSLAVFAATDG	TRVFPNKAEDWTENPEPFNASFYRRSLDNR
SEQ ID No 3	(744)	QDLNTYSLAVFAATDG	TRVFPNKAEDWTENPEPFNASFYRRSLDNR
Consensus	(751)	QDLNTYSLAVFAATDGAITRVFPNKAEDWTENPEPFNASFYRRSLDNR	850
		801	850
AF486277	(801)	GYIFKPPHQDSLRLRPLELENDTVGVLEVSTAVELSLGRRTLRLPAVVGVKLD	
SEQ ID No 3	(794)	GYIFKPPHQDSLRLRPLELENDTVGVLEVSTAVELSLGRRTLRLPAVVGVKLD	
Consensus	(801)	GYIFKPPHQDSLRLRPLELENDTVGVLEVSTAVELSLGRRTLRLPAVVGVKLD	900
		851	900
AF486277	(851)	LEAWAEKFKVLASNRTHQDQPKQCGPSSHCEMDCEVNNEDLLCVLIDDG	
SEQ ID No 3	(844)	LEAWAEKFKVLASNRTHQDQPKQCGPSSHCEMDCEVNNEDLLCVLIDDG	
Consensus	(851)	LEAWAEKFKVLASNRTHQDQPKQCGPSSHCEMDCEVNNEDLLCVLIDDG	950
		901	950
AF486277	(901)	GFLVLSNQNHQWDQVGRFFSEVDANLMLALYNNSFYTRKESYDYQAACAP	
SEQ ID No 3	(894)	GFLVLSNQNHQWDQVGRFFSEVDANLMLALYNNSFYTRKESYDYQAACAP	
Consensus	(901)	GFLVLSNQNHQWDQVGRFFSEVDANLMLALYNNSFYTRKESYDYQAACAP	1000
		951	1000
AF486277	(951)	QPPGNLGAAPRGVFPVPTIADFLNLAWWTSAAAWSLFQQLLYGLIYHSWFQ	
SEQ ID No 3	(944)	QPPGNLGAAPRGVFPVPTIADFLNLAWWTSAAAWSLFQQLLYGLIYHSWFQ	
Consensus	(951)	QPPGNLGAAPRGVFPVPTIADFLNLAWWTSAAAWSLFQQLLYGLIYHSWFQ	1050
		1001	1050
AF486277	(1001)	ADPAEAEAGSPETRESSCVMKQTQYYFGSVNASYNAIIDCGNCSRLFHAQR	
SEQ ID No 3	(994)	ADPAEAEAGSPETRESSCVMKQTQYYFGSVNASYNAIIDCGNCSRLFHAQR	
Consensus	(1001)	ADPAEAEAGSPETRESSCVMKQTQYYFGSVNASYNAIIDCGNCSRLFHAQR	1100
		1051	1100
AF486277	(1051)	LTNTNLLFVVAEKPLCSQCEVGRLLQKETHCPADGPEQCELVQRPYRRTG	
SEQ ID No 3	(1044)	LTNTNLLFVVAEKPLCSQCEVGRLLQKETHCPADGPEQCELVQRPYRRTG	
Consensus	(1051)	LTNTNLLFVVAEKPLCSQCEVGRLLQKETHCPADGPEQCELVQRPYRRTG	1150
		1101	1150
AF486277	(1101)	PHICFDYNATEDTSDCGRGASFPPSLGVLVSLQLLLLLGLPPRPQPQIHS	
SEQ ID No 3	(1094)	PHICFDYNATEDTSDCGRGASFPPSLGVLVSLQLLLLLGLPPRPQPQIHS	
Consensus	(1101)	PHICFDYNATEDTSDCGRGASFPPSLGVLVSLQLLLLLGLPPRPQPQIHS	1151
		1151	1151
AF486277	(1151)	FTPSRRL-	
SEQ ID No 3	(1144)	FAASRRL-	
Consensus	(1151)	F SRRL	

Amino acid sequence identity 98.5 %

Figure 1B

Rat brain $\alpha 2\delta$ -3 (SEQ ID NO. 6) vs rat atria $\alpha 2\delta$ -3 (AF486278)

	1	60
AF486278	(1) MAGPGLCCASRGASALLATALLYAALGDVVRSEQQIPLSVVKLWASAFGGEIKSIAAKY	
SEQ ID NO 6	(1) MAGPGLCCASRGASALLATALLYAALGDVVRSEQQIPLSVVKLWASAFGGEIKSIAAKY	
Consensus	(1) MAGPGLCCASRGASALLATALLYAALGDVVRSEQQIPLSVVKLWASAFGGEIKSIAAKY	
	61	120
AF486278	(61) SGSQLLQKKYKEYEKDVAIEEIDGLQLVKKLAKNMEEMFHKKSEAVRRLVEAAEEAHLKH	
SEQ ID NO 6	(61) SGSQLLQKKYKEYEKDVAIEEIDGLQLVKKLAKNMEEMFHKKSEAVRRLVEAAEEAHLKH	
Consensus	(61) SGSQLLQKKYKEYEKDVAIEEIDGLQLVKKLAKNMEEMFHKKSEAVRRLVEAAEEAHLKH	
	121	180
AF486278	(121) EFDADLQYEFNAVLINERDKDGNFLELGKEFILAPNDHFNNLPVNISLSDVQVPTNMYN	
SEQ ID NO 6	(121) EFDADLQYEFNAVLINERDKDGNFLELGKEFILAPNDHFNNLPVNISLSDVQVPTNMYN	
Consensus	(121) EFDADLQYEFNAVLINERDKDGNFLELGKEFILAPNDHFNNLPVNISLSDVQVPTNMYN	
	181	240
AF486278	(181) KDPAINGVVWSESLNKVFVDNFDPRPSLIWQYFGSAKGFFRQYPGIKWEPDENGVIADF	
SEQ ID NO 6	(181) KDPAINGVVWSESLNKVFVDNFDPRPSLIWQYFGSAKGFFRQYPGIKWEPDENGVIADF	
Consensus	(181) KDPAINGVVWSESLNKVFVDNFDPRPSLIWQYFGSAKGFFRQYPGIKWEPDENGVIADF	
	241	300
AF486278	(241) CRNRKWIQAATSPKDVVILVDVSGSMKGLRLTIKQTVSSILDTLGDDDFNNIITYNEE	
SEQ ID NO 6	(241) CRNRKWIQAATSPKDVVILVDVSGSMKGLRLTIKQTVSSILDTLGDDDFNNIITYNEE	
Consensus	(241) CRNRKWIQAATSPKDVVILVDVSGSMKGLRLTIKQTVSSILDTLGDDDFNNIITYNEE	
	301	360
AF486278	(301) LHYVEPCLNGTLVQADRTNKEHFREHLDKLFAKGIGMLDIALNEAFNVLSDFNHTGQGS	
SEQ ID NO 6	(301) LHYVEPCLNGTLVQADRTNKEHFREHLDKLFAKGIGMLDIALNEAFNVLSDFNHTGQGS	
Consensus	(301) LHYVEPCLNGTLVQADRTNKEHFREHLDKLFAKGIGMLDIALNEAFNVLSDFNHTGQGS	
	361	420
AF486278	(361) CSQAIMLITDGAVDYDTIFAKYNWPERKVRIFTYLIGREAAAFADNLKWMACANKGFFFTQ	
SEQ ID NO 6	(361) CSQAIMLITDGAVDYDTIFAKYNWPERKVRIFTYLIGREAAAFADNLKWMACANKGFFFTQ	
Consensus	(361) CSQAIMLITDGAVDYDTIFAKYNWPERKVRIFTYLIGREAAAFADNLKWMACANKGFFFTQ	
	421	480
AF486278	(421) ISTLADVQENVMEYLHVLSPKVIDQEHVWVTEAYIDS-----LADDQGLVLMTTVA	
SEQ ID NO 6	(421) ISTLADVQENVMEYLHVLSPKVIDQEHVWVTEAYIDSTLPQAQKLADDQGLVLMTTVA	
Consensus	(421) ISTLADVQENVMEYLHVLSPKVIDQEHVWVTEAYIDS LADDQGLVLMTTVA	
	481	540
AF486278	(475) MPVFSKQNETRSKGILLGVGTDPVKELLKTI PKYKLGINGYAFAITNNGYILTHPELR	
SEQ ID NO 6	(481) MPVFSKQNETRSKGILLGVGTDPVKELLKTI PKYKLGINGYAFAITNNGYILTHPELR	
Consensus	(481) MPVFSKQNETRSKGILLGVGTDPVKELLKTI PKYKLGINGYAFAITNNGYILTHPELR	
	541	600
AF486278	(535) PLYEKGKRRKPNYSSVDLSEVEWEDRDDVLRNAMVNRTGKFSMEVKKTVDKGRVLVM	
SEQ ID NO 6	(541) PLYEKGKRRKPNYSSVDLSEVEWEDRDDVLRNAMVNRTGKFSMEVKKTVDKGRVLVM	
Consensus	(541) PLYEKGKRRKPNYSSVDLSEVEWEDRDDVLRNAMVNRTGKFSMEVKKTVDKGRVLVM	
	601	660
AF486278	(595) TNDYYYTDIKGAPFSLGVALSRGHGKYFFRGNVTIEEGLHDLEHPDVS LADEWSYCN	
SEQ ID NO 6	(601) TNDYYYTDIKGAPFSLGVALSRGHGKYFFRGNVTIEEGLHDLEHPDVS LADEWSYCN	
Consensus	(601) TNDYYYTDIKGAPFSLGVALSRGHGKYFFRGNVTIEEGLHDLEHPDVS LADEWSYCN	
	661	720
AF486278	(655) HPEHRHLSQLEAIKLYLKGKEPLLQCDKELIQEVLFDVVSAPIEAYWTSALNKSNSD	
SEQ ID NO 6	(661) HPEHRHLSQLEAIKLYLKGKEPLLQCDKELIQEVLFDVVSAPIEAYWTSALNKSNSD	
Consensus	(661) HPEHRHLSQLEAIKLYLKGKEPLLQCDKELIQEVLFDVVSAPIEAYWTSALNKSNSD	
	721	780
AF486278	(715) KGVEVAFLGTRTGLSRINLFVGAEQLTNQDFLKARDKENIFNADHFLWYRRRAEQIPGS	
SEQ ID NO 6	(721) KGVEVAFLGTRTGLSRINLFVGAEQLTNQDFLKARDKENIFNADHFLWYRRRAEQIPGS	
Consensus	(721) KGVEVAFLGTRTGLSRINLFVGAEQLTNQDFLKARDKENIFNADHFLWYRRRAEQIPGS	
	781	840
AF486278	(775) FVYSIPFSTGTVNKSNVVTASTSIQLLDERKSPVVAAVGIQMKLEFFQRKFWTASRQCAS	
SEQ ID NO 6	(781) FVYSIPFSTGTVNKSNVVTASTSIQLLDERKSPVVAAVGIQMKLEFFQRKFWTASRQCAS	
Consensus	(781) FVYSIPFSTGTVNKSNVVTASTSIQLLDERKSPVVAAVGIQMKLEFFQRKFWTASRQCAS	
	841	900
AF486278	(835) LDGKCSISCDDDETVCYILIDNNGFILVSEDTQTGDFGEVEGAVMNKLLTMGSFKRITL	
SEQ ID NO 6	(841) LDGKCSISCDDDETVCYILIDNNGFILVSEDTQTGDFGEVEGAVMNKLLTMGSFKRITL	
Consensus	(841) LDGKCSISCDDDETVCYILIDNNGFILVSEDTQTGDFGEVEGAVMNKLLTMGSFKRITL	
	901	960
AF486278	(895) YDYQAMCRANKESSDSAHGLLDPYKAFLSAKWIVTELVLFLVEFNLCSSWWHSDMTAKAQ	
SEQ ID NO 6	(901) YDYQAMCRANKESSDSAHGLLDPYKAFLSAKWIVTELVLFLVEFNLCSSWWHSDMTAKAQ	
Consensus	(901) YDYQAMCRANKESSDSAHGLLDPYKAFLSAKWIVTELVLFLVEFNLCSSWWHSDMTAKAQ	
	961	1020

Figure 2A

```

          961                                     1020
AF486278 (955) KLKQTLEPCDTEYPAFVSERTIKETTGNACEDCSKSFVIQQIPSSNLFMVVDSSCLCE
SEQ ID NO 6 (961) KLKQTLEPCDTEYPAFVSERTIKETTGNACEDCSKSFVIQQIPSSNLFMVVDSSCLCE
Consensus (961) KLKQTLEPCDTEYPAFVSERTIKETTGNACEDCSKSFVIQQIPSSNLFMVVDSSCLCE
          1021                                     1080
AF486278 (1015) SVAPITMAPIEIRYNESLK CERLKAQKIRRRPESCHGFHPEENARECGGASSLOAQVALL
SEQ ID NO 6 (1021) SVAPITMAPIEIRYNESLK CERLKAQKIRRRPESCHGFHPEENARECGGASSLOAQVALL
Consensus (1021) SVAPITMAPIEIRYNESLK CERLKAQKIRRRPESCHGFHPEENARECGGASSLOAQVALL
          1081                                     1092
AF486278 (1075) LLPLVSSLFSR-
SEQ ID NO 6 (1081) LLPLVSSLFSR-
Consensus (1081) LLPLVSSLFSR
```

Amino Acid Identity: 99.2%

Figure 2B

Rat brain $\alpha 2\delta$ -3 splice variant (SEQ ID NO. 9) vs rat atria $\alpha 2\delta$ -3 (AF486278)

	1	60
AF486278	(1) MAGPGSLCCASRGASALLATALLYAALGDVVRSEQQIPLSVVKLWASAFGGEIKSIAAKY	
SEQ ID 9	(1) MAGPGSLCCASRGASALLATALLYAALGDVVRSEQQIPLSVVKLWASAFGGEIKSIAAKY	
Consensus	(1) MAGPGSLCCASRGASALLATALLYAALGDVVRSEQQIPLSVVKLWASAFGGEIKSIAAKY	
	61	120
AF486278	(61) SGSQLLQKKYKEYEKDVAIEEIDGLQLVKKLAKNMEEMFHKKSEAVRRLVEAAEEAHLKH	
SEQ ID 9	(61) SGSQLLQKKYKEYEKDVAIEEIDGLQLVKKLAKNMEEMFHKKSEAVRRLVEAAEEAHLKH	
Consensus	(61) SGSQLLQKKYKEYEKDVAIEEIDGLQLVKKLAKNMEEMFHKKSEAVRRLVEAAEEAHLKH	
	121	180
AF486278	(121) EFDADLQYEFYFNAVLINERDKDGNFLELGKEFILAPNDHFNNLPVNISLSDVQVPTNMYN	
SEQ ID 9	(121) EFDADLQYEFYFNAVLINERDKDGNFLELGKEFILAPNDHFNNLPVNISLSDVQVPTNMYN	
Consensus	(121) EFDADLQYEFYFNAVLINERDKDGNFLELGKEFILAPNDHFNNLPVNISLSDVQVPTNMYN	
	181	240
AF486278	(181) KDPAIVNGVWSESLNKVFVDNFRDPSLIWQYFGSAKGFFRQYPGIKWEPDENGVIADF	
SEQ ID 9	(181) KDPAIVNGVWSESLNKVFVDNFRDPSLIWQYFGSAKGFFRQYPGIKWEPDENGVIADF	
Consensus	(181) KDPAIVNGVWSESLNKVFVDNFRDPSLIWQYFGSAKGFFRQYPGIKWEPDENGVIADF	
	241	300
AF486278	(241) CRNRKWIQAATSPKDVVILVDVSGSMKGLRLTIKQTVSSILDTLGDDDDFNIIITYNEE	
SEQ ID 9	(241) CRNRKWIQAATSPKDVVILVDVSGSMKGLRLTIKQTVSSILDTLGDDDDFNIIITYNEE	
Consensus	(241) CRNRKWIQAATSPKDVVILVDVSGSMKGLRLTIKQTVSSILDTLGDDDDFNIIITYNEE	
	301	360
AF486278	(301) LHYVEPCLNGTLVQADRTNKEHFREHLDKLFAKGIGMLDIALNEAFNVLSDFNHTGQGS	
SEQ ID 9	(301) LHYVEPCLNGTLVQADRTNKEHFREHLDKLFAKGIGMLDIALNEAFNVLSDFNHTGQGS	
Consensus	(301) LHYVEPCLNGTLVQADRTNKEHFREHLDKLFAKGIGMLDIALNEAFNVLSDFNHTGQGS	
	361	420
AF486278	(361) CSQAIMLITDGAVDYDTIFAKYNWPERKVRIFTYLGREAAAFADNLKWMACANKGFFTQ	
SEQ ID 9	(361) CSQAIMLITDGAVDYDTIFAKYNWPERKVRIFTYLGREAAAFADNLKWMACANKGFFTQ	
Consensus	(361) CSQAIMLITDGAVDYDTIFAKYNWPERKVRIFTYLGREAAAFADNLKWMACANKGFFTQ	
	421	480
AF486278	(421) ISTLADVQENVMEYLHVLSRPKVIDQEHVWVTEAYIDST-----LADDQGLVLMTTVA	
SEQ ID 9	(421) ISTLADVQENVMEYLHVLSRPKVIDQEHVWVTEAYIDSTLPQAQKLADDQGLVLMTTVA	
Consensus	(421) ISTLADVQENVMEYLHVLSRPKVIDQEHVWVTEAYIDS LADDQGLVLMTTVA	
	481	540
AF486278	(475) MPVFSKQNETRSKGILLGVVGTDPVKELLKTI PKYKLGIGHYAFAITNNGYIILTHPELR	
SEQ ID 9	(481) MPVFSKQNETRSKGILLGVVGTDPVKELLKTI PKYKLGIGHYAFAITNNGYIILTHPELR	
Consensus	(481) MPVFSKQNETRSKGILLGVVGTDPVKELLKTI PKYKLGIGHYAFAITNNGYIILTHPELR	
	541	600
AF486278	(535) PLYEEGKKRRKPNYSSVDLSEVEWEDRDDVLRNAMVNRTGKFSMEVKKTVDKGRVLVM	
SEQ ID 9	(541) PLYEEGKKRRKPNYSSVDLSEVEWEDRDDVLRNAMVNRTGKFSMEVKKTVDKGRVLVM	
Consensus	(541) PLYEEGKKRRKPNYSSVDLSEVEWEDRDDVLRNAMVNRTGKFSMEVKKTVDKGRVLVM	
	601	660
AF486278	(595) TNDYYTDIKGAPFSLGVALSRHGKYYFRGNVTIEEGLHDLEHPDVSLADEWSYCNLTL	
SEQ ID 9	(601) TNDYYTDIKGAPFSLGVALSRHGKYYFRGNVTIEEGLHDLEHPDVSLADEWSYCNLTL	
Consensus	(601) TNDYYTDIKGAPFSLGVALSRHGKYYFRGNVTIEEGLHDLEHPDVSLADEWSYCNLTL	
	661	720
AF486278	(655) HPEHRHLSQLEAIKLYLKGEPLQCDKELIQEVLFDVAVVSAPIEAYWTSALNKSNSD	
SEQ ID 9	(661) HPEHRHLSQLEAIKLYLKGEPLQCDKELIQEVLFDVAVVSAPIEAYWTSALNKSNSD	
Consensus	(661) HPEHRHLSQLEAIKLYLKGEPLQCDKELIQEVLFDVAVVSAPIEAYWTSALNKSNSD	
	721	780
AF486278	(715) KGVEVAFLGTRTGLSRINLFVGAEQLTNQDFLKARDKENIFNADHFPLWYRRAEQIPGS	
SEQ ID 9	(721) KGVEVAFLGTRTGLSRINLFVGAEQLTNQDFLKARDKENIFNADHFPLWYRRAEQIPGS	
Consensus	(721) KGVEVAFLGTRTGLSRINLFVGAEQLTNQDFLKARDKENIFNADHFPLWYRRAEQIPGS	
	781	840
AF486278	(775) FVYSIPFSTGTVNKSNVVTASTSIQLLDERKSPVVAAGVIQMKLEFFQRKFWTASRQCAS	
SEQ ID 9	(781) FVYSIPFSTGTVNKSNVVTASTSIQLLDERKSPVVA-----	
Consensus	(781) FVYSIPFSTGTVNKSNVVTASTSIQLLDERKS	
	841	900
AF486278	(835) LDGKCSISCDDETVCNYLIDNNGFILVSEDYTOTGDFFEVEGAVMNKLLTMGSFKRITL	
SEQ ID 9	(813) -----	
Consensus	(841) -----	
	901	960
AF486278	(895) YDYQAMCRANKESSDSAHLDPYKAFLSAKWIVTELVLFLVEFNLCSSWWHSDMTAKAQ	
SEQ ID 9	(814) -----AQ	
Consensus	(901) -----AQ	
	961	1020

Figure 3A

```

          961                                     1020
AF486278 (955) KKKQTLEPCDTEYPAFVSERTIKETTGNIACEDCSKSFVIQQIPSSNLFMVVDSSCLCE
SEQ ID 9 (819) KKKQTLEPCDTEYPAFVSERTIKETTGNIACEDCSKSFVIQQIPSSNLFMVVDSSCLCE
Consensus (961) KKKQTLEPCDTEYPAFVSERTIKETTGNIACEDCSKSFVIQQIPSSNLFMVVDSSCLCE
          1021                                     1080
AF486278 (1015) SVAPITMAPIEIRYNESLK CERLKAQKIRRRPESCHGFHPEENARECGGASSLQAQVALL
SEQ ID 9 (879) SVAPITMAPIEIRYNESLK CERLKAQKIRRRPESCHGFHPEENARECGGASSLQAQVALL
Consensus (1021) SVAPITMAPIEIRYNESLK CERLKAQKIRRRPESCHGFHPEENARECGGASSLQAQVALL
          1081      1091
AF486278 (1075) LLPLVSSLFSR
SEQ ID 9 (939) LLPLVSSLFSR
Consensus (1081) LLPLVSSLFSR

```

Sequence identity 86.0%

Figure 3B

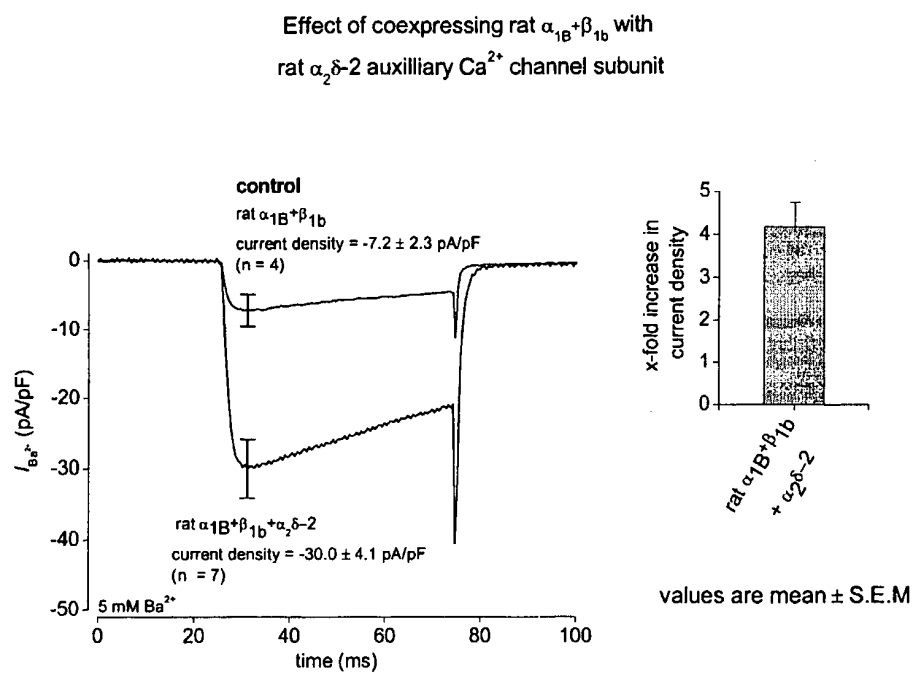


Figure 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/001550

A. CLASSIFICATION OF SUBJECT MATTER

C12N 15/12 C07K 14/705 C07K 14/47 C12N 5/10 C12P 21/02 C12Q 1/02 C12Q 1/68 G01N 33/566 G01N 33/53
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)
C12N C07K C12Q G01N

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 practicable, search terms used)
GeneSeq, GenBank (incl. EST and HTG), PIR, SwissProt, DGene, Delphion, CAPLUS, Biosis, Medline, PubMed
Keywords: calcium channel and rat and brain isoform

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	CHU, P.J. and BEST, P. M. "Molecular cloning of calcium channel $\alpha_2\delta$ -subunits from rat atria and the differential regulation of their expression by IGF-1". J MOL CELL CARDIOL. February 2003, Vol. 35, No. 2, pages 207-215, whole document, especially figures 1 and 3.	1 to 7 8 to 10, 13, 14, 17 to 23
X Y	KLUGBAUER, N., et. al. "Molecular diversity of the calcium channel $\alpha_2\delta$ subunit". J NEUROSCIENCE. 15 January 1999, Vol. 19, No. 2, pages 684-691, whole document.	1 to 7, 13, 14 8 to 10, 19 to 23
X Y	WO 01/19870 A2 (BROWN, J. and BERTELLI, F.) 22 March 2001, whole document.	1 to 7, 11, 12 8 to 10, 17, 18, 23
X	WO 03/062401 A2 (GAIGER et. al.) 31 July 2003, sequence ID number 9.	15, 16

Further documents are listed in the continuation of Box C.

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 application or patent 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-type search
10 December 2004 (10-12-2004)Date of mailing of the international-type search report
07 January 2005 (07-01-2005)

Name and mailing address of the ISA/CA
Commissioner of Patents
Canadian Patent Office - PCT
Ottawa/Gatineau K1A 0C9
Facsimile No. 1-819-953-9358

Authorized officer
Kathleen Pound (819) 953-9757

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2004/001550

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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. ☐ Claims Nos. :
because they relate to subject matter not required to be searched by this Authority; namely:

2. ☐ Claims Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

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

Box III Observation where unity of invention is lacking (Continuation of item 3 of first sheet)

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

1. Claims 1 to 10 (as applicable), 11, 12, 17, 18 and 23 (as applicable) pertaining to isolated nucleic acids of the rat brain $\alpha_2\delta$ -2 calcium channel subunit (sequence ID numbers 2 and 3) and splice variants thereof (sequence ID numbers 10 and 11), corresponding proteins, cells, probes, methods and antibodies.
 2. Claims 1 to 10 (as applicable), 13 to 16, 19 to 22 and 23 (as applicable) pertaining to isolated nucleic acids of the rat brain $\alpha_2\delta$ -3 calcium channel subunit (sequence ID numbers 5 and 6) and splice variants thereof (sequence ID numbers 8 and 9), corresponding proteins, cells, probes, methods and antibodies.
- The technical feature shared between these two groups is that they are calcium channel subunits from rat brain. Rat calcium channel subunits of both $\alpha_2\delta$ -2 and $\alpha_2\delta$ -3 types are known from rat atria (GenBank™ accession numbers AF486277 and AF486278). Moreover, $\alpha_2\delta$ -3 and $\alpha_2\delta$ -2 subunits are known to exist in rodent and human brain tissue, respectively (Klugbauer et al., J. Neuroscience, 19: 684-691). Consequently there is no special unifying technical feature shared by these two claim groupings and unity of invention is lacking.

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
Information on patent family members

International application No.
PCT/CA2004/001550

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date	
WO0119870 A2	22-03-2001	AU7420100	A	17-04-2001
		BR0014068	A	21-05-2002
		CA2385308	A1	22-03-2001
		EP1214349	A2	19-06-2002
		JP2003511011T	T	25-03-2003
		US6783952	B1	31-08-2004
		WO0119870	A2	22-03-2001
		WO03062401 A2	31-07-2003	AU4347701
CA2463659	A1			25-09-2003
CA2473746	A1			31-07-2003
EP1474510	A2			10-11-2004
JP2002231951	A			16-08-2002
US2002177260	A1			28-11-2002
US2002198362	A1			26-12-2002
US2003078396	A1			24-04-2003
US2004002068	A1			01-01-2004
US2004005561	A1			08-01-2004
US2004175739	A1			09-09-2004
WO0164886	A2			07-09-2001
WO03062401	A2			31-07-2003
WO03077836	A2			25-09-2003