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(73) Patenthaver: **Teva Pharmaceuticals International GmbH, Schlüsselstrasse 12, 8645 Jona, Schweiz**

(72) Opfinder: **ZELLER, Joerg, 1002 Sunnyside Boulevard, Ann Arbor, MI Michigan 48103, USA**
ABDICHE, Yasmina Noubia, 183 Ada Avenue, Unit A, Mountain View, CA California 94043, USA
PONS, Jaume, 1723 Hickory Avenue, San Bruno, CA California 94066, USA
ROSENTHAL, Arnon, 150 Normandy Lane, Woodside, CA California 94062, USA
COLLIER, Sierra Jones, 521 - 7th Avenue, Menlo Park, CA California 94025, USA
POULSEN, Kristian Todd, 2080 Third Street, No. 7, San Francisco, CA 94107, USA

(74) Fuldmægtig i Danmark: **Plougmann Vingtoft A/S, Rued Langgaards Vej 8, 2300 København S, Danmark**

(54) Benævnelse: **ANTAGONISTANTISTOFFER RETTET MOD CALCITONIN GEN-RELATERET PEPTID TIL BEHANDLING AF KLYNGE-HOVEDPINE**

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DESCRIPTION

Field of the Invention

[0001] The present invention relates to the use of anti-CGRP antagonist antibodies for the prevention, amelioration, or treatment of vasomotor symptoms, such as CGRP related headaches (e.g., migraine) and hot flushes.

Background of the Invention

[0002] CGRP (calcitonin gene-related peptide) is a 37 amino acid neuropeptide, which belongs to a family of peptides that includes calcitonin, adrenomedullin and amylin. In humans, two forms of CGRP (a-CGRP and β-CGRP) exist and have similar activities. They vary by three amino acids and exhibit differential distribution. At least two CGRP receptor subtypes may also account for differential activities. CGRP is a neurotransmitter in the central nervous system, and has been shown to be a potent vasodilator in the periphery, where CGRP-containing neurons are closely associated with blood vessels. CGRP-mediated vasodilatation is also associated with neurogenic inflammation, as part of a cascade of events that results in extravasation of plasma and vasodilation of the microvasculature and is present in migraine.

[0003] CGRP has been noted for its possible connection to vasomotor symptoms (Wyon et al. Scand. J. Urol. Nephrol. 35: 92-96 (2001); Wyon et al. Menopause 7(1):25-30 (2000)). Vasomotor symptoms (VMS), such as hot flushes and night sweats, are the most common symptoms associated with menopause, occurring in 60% to 80% of all women following natural or surgically-induced menopause. Hot flushes are likely to be an adaptive response of the central nervous system (CNS) to declining sex steroids (Freedman Am. J. Human Biol. 13:453-464 (2001)). To date, the most effective therapies for flushes are hormone-based treatments, including estrogens and/or some progestins. Hormonal treatments can be effective for alleviating flushes, but are not appropriate for all women. Psychological and emotional symptoms observed, such as nervousness, fatigue, irritability, insomnia, depression, memory loss, headache, anxiety, nervousness or inability to concentrate are considered to be caused by the sleep deprivation following hot flush and night sweats (Kramer et al., In: Murphy et al., 3.sup.rd Int'l Symposium on Recent Advances in Urological Cancer Diagnosis and Treatment-Proceedings, Paris, France: SCI: 3-7 (1992)).

[0004] Men also experience hot flushes following steroid hormone (androgen) withdrawal. This is true in cases of age-associated androgen decline (Katovich, et al., Proceedings of the Society for Experimental Biology & Medicine, 1990, 193(2): 129-35) as well as in extreme cases of hormone deprivation associated with treatments for prostate cancer (Berendsen, et al., European Journal of Pharmacology, 2001, 419(1): 47-54). As many as one-third of these patients will experience persistent and frequent symptoms severe enough to cause significant

discomfort and inconvenience.

[0005] CGRP is a potent vasodilator that has been implicated in the pathology of other vasomotor symptoms, such as all forms of vascular headache, including migraines (with or without aura) and cluster headache. Durham, N. Engl. J. Med. 350:1073-1075, 2004. The serum levels of CGRP in the external jugular vein are elevated in patients during migraine headache. Goadsby et al., Ann. Neurol. 28:183-7, 1990. Intravenous administration of human α-CGRP induced headache and migraine in patients suffering from migraine without aura, suggesting that CGRP has a causative role in migraine. Lassen et al., Cephalalgia 22:54-61, 2002.

[0006] Possible CGRP involvement in migraine has been the basis for the development and testing of a number of compounds that inhibit release of CGRP (e.g., sumatriptan), antagonize at the CGRP receptor (e.g., dipeptide derivative BIBN4096BS (Boerhringer Ingelheim); CGRP(8-37)), or interact with one or more of receptor-associated proteins, such as, receptor activity membrane protein (RAMP) or receptor component protein (RCP), both of which affect binding of CGRP to its receptors. Brain, S. et al., Trends in Pharmacological Sciences 23:51-53, 2002. Alpha-2 adrenoceptor subtypes and adenosine A1 receptors also control (inhibit) CGRP release and trigeminal activation (Goadsby et al., Brain 125:1392-401, 2002). The adenosine A1 receptor agonist GR79236 (metrafadil), which has been shown to inhibit neurogenic vasodilation and trigeminal nociception in humans, may also have anti-migraine activity (Arulmani et al., Cephalalgia 25:1082-1090, 2005; Giffin et al., Cephalalgia 23:287-292, 2003.)

[0007] Confounding this theory is the observation that treatment with compounds that exclusively inhibit neurogenic inflammation (e.g., tachykinin NK1 receptor antagonists) or trigeminal activation (e.g., $5HT_{1D}$ receptor agonists) have been shown to be relatively ineffective as acute treatments for migraine, leading some investigators to question whether inhibiting release of CGRP is the primary mechanism of action of effective anti-migraine treatments. Arulmani et al., Eur. J. Pharmacol. 500:315-330, 2004.

[0008] Migraine is a complex, common neurological condition that is characterized by severe, episodic attacks of headache and associated features, which may include nausea, vomiting, sensitivity to light, sound or movement. In some patients, the headache is preceded or accompanied by an aura. The headache pain may be severe and may also be unilateral in certain patients.

[0009] Migraine attacks are disruptive to daily life. In US and Western Europe, the overall prevalence of migraine sufferers is 11% of the general population (6% males; 15-18% females). Furthermore, the median frequency of attacks in an individual is 1.5/month. While there are a number of treatments available to alleviate or reduce symptoms, preventive therapy is recommended for those patients having more than 3-4 attacks of migraine per month. Goadsby et al. New Engl. J. Med. 346(4): 257-275, 2002.

[0010] The variety of pharmacologic interventions that have been used to treat migraine and the variability in responses among patients are a testament to the diverse nature of this disorder. Thus, such relatively non-selective drugs as ergot alkaloids (e.g., ergotamine, dihydroergotamine, methysergide), which exhibit serotonergic, as well as adrenergic, noradrenergic and dopaminergic activity, have been used for over eighty years to treat migraine. Other treatments include opiates (e.g., oxycodone) and β-adrenergic antagonists (e.g., propranolol). Some patients, usually those with milder symptoms, are able to control their symptoms with non-prescription remedies such as one or more non-steroidal anti-inflammatory agents (NSAIDs), such as a combination of aspirin, acetaminophen and caffeine (e.g., Excedrin® Migraine).

[0011] More recently, some migraine patients have been treated with topiramate, an anticonvulsant that blocks voltage-dependent sodium channels and certain glutamate receptors (AMPA-kainate), potentiates GABA-A receptor activity, and blocks carbonic anhydrase. The relatively recent success of serotonin 5HT-1B/1D and/or 5HT-1a receptor agonists, such as sumatriptan, in some patients has led researchers to propose a serotonergic etiology of the disorder. Unfortunately, while some patients respond well to this treatment, others are relatively resistant to its effects.

[0012] It has been postulated that a dysfunction of an ion channel in the aminergic brainstem nuclei underlies the disorder, however, the precise pathophysiology of migraine is not yet well understood. One form of migraine, familial hemiplagic migraine, has been shown to associated with missense mutations in the α1 subunit of the voltage-gated P/Q-type calcium channel, and it is thought likely that other ion-channel mutations will also be found in other populations of patients. While dilation of blood vessels is associated with and exacerbates the pain symptoms of migraine, such neurovascular events are now thought to be a result of, rather than causative of, the condition. Overall, dysfunction of brainstem pathways modulating sensory input is considered to be a unifying feature of migraine. Goadsby, P.J. et al., New Engl. J. Med. 346(4): 257-275, 2002.

[0013] US2005/0234054 discloses small molecule CGRP receptor antagonists for use in treating a variety of diseases, including cluster headache. Tan et al. discusses the role of CGRP as a potent vasodilator. The authors show that administration of an anti-CGRP Fab fragment blocked the increased skin blood flow response to antidromic stimulation of the saphenous nerve in rats. Furthermore, Tan et al. indicates that the block of vasodilation is not achieved to the same extent by a full length anti-CGRP antibody.

Brief Summary of the Invention

[0014] The invention disclosed herein concerns methods of using anti-CGRP antagonist antibodies for treating or preventing cluster headaches

[0015] In accordance with the present invention, there is provided an anti-CGRP antagonist

antibody for use in the prevention or treatment of cluster headache.

[0016] In one aspect, the present invention provides an anti-CGRP antagonist antibody for use in a method for treating or preventing cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody.

[0017] In one aspect, the present invention provides an anti-CGRP antagonist antibody for use in a method for treating or preventing cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody.

[0018] In another aspect, the invention provides an anti-CGRP antagonist antibody for use in a method for ameliorating, controlling, reducing incidence of, or delaying the development or progression of cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody.

[0019] In a further embodiment, the invention provides an anti-CGRP antagonist antibody for use in methods for ameliorating, controlling, reducing incidence of, or delaying the development or progression of cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody in combination with at least one additional agent useful for treating headache. Such additional agents include 5-HT1-like agonists (and agonists acting at other 5-HT1 sites), and non-steroidal anti-inflammatory drugs (NSAIDs).

[0020] Examples of 5-HT1 agonists that can be used in combination with an anti-CGRP antibody include a class of compounds known as triptans, such as sumatriptan, zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, and frovatriptan. Ergot alkaloids and related compounds are also known to have 5-HT agonist activity and have been used to treat headache such as migraine. Included among these compounds are ergotamine tartrate, ergonovine maleate, and ergoloid mesylates (e.g., dihydroergocornine, dihydroergocristine, dihydroergocryptine, and dihydroergotamine mesylate (DHE 45)).

[0021] Examples of NSAIDs that can be used in combination with an anti-CGRP antibody include naproxen, flurbiprofen, ketoprofen, oxaprozin, etodolac, indomethacin, ketorolac, nabumetone, mefanamic acid, and piroxican. Additional NSAIDs include cyclooxygenase-2 (COX-2) inhibitors. Members of this group include: celecoxib; rofecoxib; meloxicam; JTE-522; L-745,337; NS398; and pharmaceutically acceptable salts thereof.

[0022] In some embodiments, the anti-CGRP antagonist antibody recognizes a human CGRP. In some embodiments, the anti-CGRP antagonist antibody binds to both human α-CGRP and β-CGRP. In some embodiments, the anti-CGRP antagonist antibody binds human and rat CGRP. In some embodiments, the anti-CGRP antagonist antibody binds the C-terminal fragment having amino acids 25-37 of CGRP. In some embodiments, the anti-CGRP antagonist antibody binds a C-terminal epitope within amino acids 25-37 of CGRP.

[0023] In some embodiments, the anti-CGRP antagonist antibody is a monoclonal antibody. In some embodiments, the anti-CGRP antagonist antibody is humanized. In some embodiments, the antibody is human. In some embodiments, the anti-CGRP antagonist antibody is antibody G1 (as described herein). In some embodiments, the anti-CGRP antagonist antibody comprises one or more CDR(s) (such as one, two, three, four, five, or, in some embodiments, all six CDRs) of antibody G1 or variants of G1 shown in Table 6. In still other embodiments, the anti-CGRP antagonist antibody comprises the amino acid sequence of the heavy chain variable region shown in Figure 5 (SEQ ID NO: 1) and the amino acid sequence of the light chain variable region shown in Figure 5 (SEQ ID NO: 2).

[0024] In some embodiments, the antibody comprises a modified constant region, such as a constant region that is immunologically inert (including partially immunologically inert), e.g., does not trigger complement mediated lysis, does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC), does not activate microglia, or having reduced one or more of these activities. In some embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; and WO 99/58572. In other embodiments, the antibody comprises a human heavy chain IgG2 constant region comprising the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence). Eur. J. Immunol. (1999) 29:2613-2624. In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG1 with any of the following mutations: 1) A327A330P331 to G327S330S331; 2) E233L234L235G236 to P233V234A235 with G236 deleted; 3) E233L234L235 to P233V234A235; 4) E233L234L235G236A327A330P331 to P233V234A235G327S330S331 with G236 deleted; 5) E233L234L235A327A330P331 to P233V234A235G327S330S331; and 6) N297 to A297 or any other amino acid except N. In some embodiments, the heavy chain constant region of the antibody is a human heavy chain IgG4 with any of the following mutations: E233F234L235G236 to P233V234A235 with G236 deleted; E233F234L235 to P233V234A235; and S228L235 to P228E235.

[0025] In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the oligosaccharide attachment residue (such as Asn297) and/or flanking residues that are part of the N-glycosylation recognition sequence in the constant region. In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked glycosylation enzymatically or by expression in a glycosylation deficient host cell.

[0026] The binding affinity (K_D) of an anti-CGRP antagonist antibody to CGRP (such as human α-CGRP as measured by surface plasmon resonance at an appropriate temperature, such as 25 or 37 °C) can be about 0.02 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, or about 2 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM.

[0027] The anti-CGRP antagonist antibody may be administered prior to, during and/or after headache. In some embodiments, the anti-CGRP antagonist antibody is administered prior to the attack of cluster headache. Administration of an anti-CGRP antagonist antibody can be by any means known in the art, including: orally, intravenously, subcutaneously, intraarterially, intramuscularly, intracardially, intraspinally, intrathoracically, intraperitoneally, intraventricularly, sublingually, transdermally, and/or via inhalation. Administration may be systemic, e.g. intravenously, or localized.

[0028] In some embodiments, the anti-CGRP antagonist antibody may be administered in conjunction with an another agent, such as another agent for treating headache.

[0029] In another aspect, the invention provides use of an anti-CGRP antagonist antibody for the manufacture of a medicament for use in any of the methods described herein, for example, for treating or preventing headache.

[0030] The invention can use anti-CGRP antagonist antibodies derived from antibody G1 or its variants shown in Table 6. Antibody G1 (interchangeably termed "G1") is produced by expression vectors having ATCC Accession Nos. PTA-6866 and PTA-6867. The amino acid sequences of the heavy chain and light chain variable regions of G1 are shown in Figure 5. The complementarity determining region (CDR) portions of antibody G1 (including Chothia and Kabat CDRs) are also shown in Figure 5. It is understood that reference to any part of or entire region of G1 encompasses sequences produced by the expression vectors having ATCC Accession Nos. PTA-6866 and PTA-6867, and/or the sequences depicted in Figure 5. Antibody variants of G1 with amino acid sequences depicted in Table 6 can also be used.

[0031] An antibody useful in the invention can comprise a V_H domain that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% at least 98%, at least 99% or 100% identical in amino acid sequence to SEQ ID NO: 1.

[0032] An antibody useful in the invention can comprise a V_L domain that is at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97% at least 98%, at least 99% or 100% identical in amino acid sequence to SEQ ID NO: 2.

[0033] An antibody useful in the invention can comprise a fragment or a region of the antibody G1 or its variants shown in Table 6. In one embodiment, the fragment is a light chain of the antibody G1. In another embodiment, the fragment is a heavy chain of the antibody G1. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain of the antibody G1. In yet another embodiment, the fragment contains one or more variable regions from a light chain and/or a heavy chain shown in Figure 5. In yet another embodiment, the fragment contains one or more CDRs from a light chain and/or a heavy chain of the antibody G1.

[0034] The invention can use an antibody comprising a V_H CDR3 as set forth in SEQ ID NO: 5, or a sequence that differs from SEQ ID NO: 5 by 1, 2, 3, 4, or 5 amino acid substitutions. In a particular embodiment, such amino acid substitutions are conservative substitutions.

[0035] The invention can use an antibody comprising a V_L CDR3 as set forth in SEQ ID NO: 8, or a sequence that differs from SEQ ID NO: 8 by 1, 2, 3, 4, or 5 amino acid substitutions. In a particular embodiment, such amino acid substitutions are conservative substitutions.

[0036] The invention can use an antibody comprising any one or more of the following: a) one or more CDR(s) of antibody G1 or its variants shown in Table 6; b) CDR H3 from the heavy chain of antibody G1 or its variants shown in Table 6; c) CDR L3 from the light chain of antibody G1 or its variants shown in Table 6; d) three CDRs from the light chain of antibody G1 or its variants shown in Table 6; e) three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6; f) three CDRs from the light chain and three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6. The invention can use an antibody comprising any one or more of the following: a) one or more (one, two, three, four, five, or six) CDR(s) derived from antibody G1 or its variants shown in Table 6; b) a CDR derived from CDR H3 from the heavy chain of antibody G1; and/or c) a CDR derived from CDR L3 from the light chain of antibody G1. In some embodiments, the CDR is a CDR shown in Figure 5. In some embodiments, the one or more CDRs derived from antibody G1 or its variants shown in Table 6 are at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identical to at least one, at least two, at least three, at least four, at least five, or at least six CDRs of G1 or its variants.

[0037] In some embodiments, the CDR is a Kabat CDR. In other embodiments, the CDR is a Chothia CDR. In other embodiments, the CDR is a combination of a Kabat and a Chothia CDR (also termed "combined CDR" or "extended CDR"). In other words, for any given embodiment containing more than one CDR, the CDRs may be any of Kabat, Chothia, and/or combined.

[0038] In some embodiments, the antibody comprises the amino acid sequence of KASKXaaVXaaTYVS, wherein Xaa at position 5 is R, W, G, L, or N; and wherein Xaa at position 7 is T, A, D, G, R, S, W, or V. In some embodiments, the amino acid sequence of KASKXaaVXaaTYVS is CDR1 of an antibody light chain.

[0039] In some embodiments, the antibody comprises the amino acid sequence of XaaXaaSNRYXaa, wherein Xaa at position 1 is G or A; wherein Xaa at position 2 is A or H; and wherein Xaa at position 7 is L, T, I, or S. In some embodiments, the amino acid sequence of XaaXaaSNRYXaa is CDR2 of an antibody light chain.

[0040] In some embodiments, the antibody comprises the amino acid sequence of EIRSXaaSDXaaXaaATXaaYAXaaAVKG, wherein Xaa at position 5 is E, R, K, Q, or N; wherein

Xaa at position 8 is A, G, N, E, H, S, L, R, C, F, Y, V, D, or P; wherein Xaa at position 9 is S, G, T, Y, C, E, L, A, P, I, N, R, V, D, or M; wherein Xaa at position 12 is H or F; wherein Xaa at position 15 is E or D. In some embodiments, the amino acid sequence of EIRSXaaSDXaaXaaATXaaYAXaaAVKG is CDR2 of an antibody heavy chain.

[0041] In some embodiments, the antibody comprises the amino acid sequence of SEQ ID NO:1, wherein amino acid residue at position 99 of SEQ ID NO:1 is L or is substituted by A, N, S, T, V, or R; and wherein amino acid residues at position 100 of SEQ ID NO:1 is A or is substituted by L, R, S, V, Y, C, G, T, K, or P.

[0042] In some embodiments, the antibody is a human antibody. In other embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is monoclonal. In some embodiments, the antibody (or polypeptide) is isolated. In some embodiments, the antibody (or polypeptide) is substantially pure.

[0043] The heavy chain constant region of the antibodies may be from any types of constant region, such as IgG, IgM, IgD, IgA, and IgE; and any isotypes, such as IgG1, IgG2, IgG3, and IgG4.

[0044] In some embodiments, the antibody comprises a modified constant region as described herein.

[0045] The present invention uses anti-CGRP antagonist antibodies for treating, preventing, ameliorating, controlling, or reducing incidence of cluster headache.

Brief Description of the Drawings

[0046]

Figure 1 is a table showing binding affinities of 12 murine antibodies for different alanine substituted human α-CGRP fragments. Binding affinities were measured at 25°C using Biacore by flowing Fabs across CGRPs on the chip. The boxed values represent the loss in affinity of alanine mutants relative to parental fragment, 25-37 (italic), except K35A, which was derived from a 19-37 parent. "a" indicates affinities for 19-37 and 25-37 fragments are the mean average ± standard deviation of two independent measurements on different sensor chips. "b" indicates these interactions deviated from a simple bimolecular interaction model due to a biphasic offrate, so their affinities were determined using a conformational change model. Greyscale key: white (1.0) indicates parental affinity; light grey (less than 0.5) indicates higher affinity than parent; dark grey (more than 2) indicates lower affinity than parent; and black indicates that no binding was detected.

Figures 2A and 2B show the effect of administering CGRP 8-37 (400 nmol/kg), antibody 4901 (25 mg/kg), and antibody 7D11 (25 mg/kg) on skin blood flow measured as blood cell flux after

electrical pulse stimulation for 30 seconds. CGRP 8-37 was administered intravenously (iv) 3-5 min before electrical pulse stimulation. Antibodies were administered intraperitoneal (IP) 72 hours before electrical pulse stimulation. Each point in the graphs represents AUC of one rat treated under the conditions as indicated. Each line in the graphs represents average AUC of rats treated under the condition as indicated. AUC (area under the curve) equals to Δflux x Δtime. "Δflux" represents the change of flux units after the electrical pulse stimulation; and "Δtime" represents the time period taken for the blood cell flux level to return to the level before the electrical pulse stimulation.

Figure 3 shows the effect of administering different dosage of antibody 4901 (25 mg/kg, 5 mg/kg, 2.5 mg/kg, or 1 mg/kg) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. Antibodies were administered intravenously (IV) 24 hours before electrical pulse stimulation. Each point in the graph represents AUC of one rat treated under the conditions as indicated. The line in the graph represents average AUC of rats treated under the condition as indicated.

Figures 4A and 4B show the effect of administering antibody 4901 (1 mg/kg or 10 mg/kg, i.v.), antibody 7E9 (10 mg/kg, i.v.), and antibody 8B6 (10 mg/kg, i.v.) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. Antibodies were administered intravenously (i.v.) followed by electrical pulse stimulation at 30 min, 60 min, 90 min, and 120 min after antibody administration. Y axis represents percent of AUC as compared to level of AUC when no antibody was administered (time 0). X axis represents time (minutes) period between the administration of antibodies and electrical pulse stimulation. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to time 0. Data were analyzed using one-way ANOVA with a Dunnett's Multiple comparison test.

Figure 5 shows the amino acid sequence of the heavy chain variable region (SEQ ID NO:1) and light chain variable region (SEQ ID NO:2) of antibody G1. The Kabat CDRs are in bold text, and the Chothia CDRs are underlined. The amino acid residues for the heavy chain and light chain variable region are numbered sequentially.

Figure 6 shows epitope mapping of antibody G1 by peptide competition using Biacore. N-biotinylated human α-CGRP was captured on SA sensor chip. G1 Fab (50 nM) in the absence of a competing peptide or pre-incubated for 1 h with 10 uM of a competing peptide was flowed onto the chip. Binding of G1 Fab to the human α-CGRP on the chip was measured. Y axis represents percentage of binding blocked by the presence of the competing peptide compared with the binding in the absence of the competing peptide.

Figure 7 shows the effect of administering antibody G1 (1 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds. Antibody G1 or vehicle was administered intravenously (i.v.) followed by nerve electrical pulse stimulation at 30 min, 60 min, 90 min, and 120 min after antibody administration. Y axis represents percent of AUC as compared to level of AUC when no antibody or vehicle (defined as 100%) was administered (time 0). X axis represents time (minutes) period between the administration of antibodies and electrical pulse stimulation. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to vehicle. Data were analyzed

using two-way ANOVA and Bonferroni post tests.

Figure 8A shows the effect of administering antibody G1 (1 mg/kg, 3 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds 24 hours after dosing. Antibody G1 or vehicle was administered intravenously (i.v.) 24 hours before nerve electrical pulse stimulation. Y axis represents total area under curve (change in blood cell flux multiplied by the change in time from stimulation until flux returns to baseline, AUC). X axis represents varying doses of antibody G1. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to vehicle. Data were analyzed using one-way ANOVA and Dunn's multiple comparison test.

Figure 8B shows the effect of administering antibody G1 (0.3 mg/kg, 1 mg/kg, 3 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on skin blood flow measured as blood cell flux after electrical pulse stimulation for 30 seconds 7 days after dosing. Antibody G1 or vehicle was administered intravenously (i.v.) 7 days before nerve electrical pulse stimulation. Y axis represents total AUC. X axis represents varying doses of antibody G1 . "**" indicates $P < 0.01$, and "***" indicates $P < 0.001$, as compared to vehicle. Data were analyzed using one-way ANOVA and Dunn's multiple comparison test.

Figure 8C is a curve fit analysis of the data from figures 8A and 8B. Antibody G1 or vehicle was administered intravenously (i.v.) either 24 hours or 7 days before nerve electrical pulse stimulation. Y axis represents total AUC. X axis represents varying doses of antibody G1 in "mg/kg" on a logarithmic scale to determine EC_{50}.

Figure 9 shows the effect of antibody mu7E9 (10 mg/kg), BIBN4096BS or vehicle (PBS, 0.01% Tween 20) on the change in diameter of the middle meningeal artery after electrical field stimulation. Antibody mu7E9, BIBN4096BS or vehicle were administered intravenously (i.v.) at time point 0 minutes after a baseline response to electrical stimulation was established. Y axis represents change in diameter of the middle meningeal artery after electrical field stimulation. Resting diameter corresponds to 0%. X axis represents time (minutes) of electrical pulse stimulation. "*" indicates $P < 0.05$, and "**" indicates $P < 0.01$, as compared to vehicle. Data were analyzed using one-way ANOVA and Dunett's multiple comparison test.

Figure 10 shows the effect of varying doses of antibody G1 (1 mg/kg, 3 mg/kg or 10 mg/kg, i.v.) or vehicle (PBS, 0.01% Tween 20) on the change in diameter of the middle meningeal artery after electrical field stimulation. Antibody G1 or vehicle was administered intravenously (i.v.) 7 days before electrical field stimulation. Y axis represents change in diameter of the middle meningeal artery. Resting diameter corresponds to 0%. X axis represents stimulation voltage. "*" indicates $P < 0.05$, "**" indicates $P < 0.01$, and "***" indicates $P < 0.001$, as compared to vehicle. Data were analyzed using two-way ANOVA and Bonferroni posttests.

Figure 11A shows the effect of antibody mu4901 (10mg/kg) or vehicle (PBS, 0.01% Tween 20), administered intravenously (i.v.) 24 hours prior, on the decrease in core temperature induced by subcutaneous injection of naloxone (1mg/kg) in morphine addicted rats. The Y axis represents temperature difference from baseline. The X axis represents time measured from the point of naloxone injection.

Figure 11B shows the effect of antibody mu4901 (10mg/kg) or vehicle (PBS, 0.01% Tween 20), administered intravenously (i.v.) 24 hours prior, on the increase in tail surface temperature induced by subcutaneous injection of naloxone (1mg/kg) in morphine addicted rats. The Y axis represents temperature difference from baseline. The X axis represents time measured from the point of naloxone injection.

Detailed Description of the Invention

[0047] The invention disclosed herein provides methods for treating and/or preventing cluster headache in an individual by administering to the individual a therapeutically effective amount of an anti-CGRP antagonist antibody.

[0048] Anti-CGRP antagonist antibodies derived from G1 or its variants shown in Table 6 can also be used.

General Techniques

[0049] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are within the skill of the art. Such techniques are explained fully in the literature, such as, Molecular Cloning: A Laboratory Manual, second edition (Sambrook et al., 1989) Cold Spring Harbor Press; Oligonucleotide Synthesis (M.J. Gait, ed., 1984); Methods in Molecular Biology, Humana Press; Cell Biology: A Laboratory Notebook (J.E. Cellis, ed., 1998) Academic Press; Animal Cell Culture (R.I. Freshney, ed., 1987); Introduction to Cell and Tissue Culture (J.P. Mather and P.E. Roberts, 1998) Plenum Press; Cell and Tissue Culture: Laboratory Procedures (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-1998) J. Wiley and Sons; Methods in Enzymology (Academic Press, Inc.); Handbook of Experimental Immunology (D.M. Weir and C.C. Blackwell, eds.); Gene Transfer Vectors for Mammalian Cells (J.M. Miller and M.P. Calos, eds., 1987); Current Protocols in Molecular Biology (F.M. Ausubel et al., eds., 1987); PCR: The Polymerase Chain Reaction, (Mullis et al., eds., 1994); Current Protocols in Immunology (J.E. Coligan et al., eds., 1991); Short Protocols in Molecular Biology (Wiley and Sons, 1999); Immunobiology (C.A. Janeway and P. Travers, 1997); Antibodies (P. Finch, 1997); Antibodies: a practical approach (D. Catty., ed., IRL Press, 1988-1989); Monoclonal antibodies: a practical approach (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); Using antibodies: a laboratory manual (E. Harlow and D. Lane (Cold Spring Harbor Laboratory Press, 1999); The Antibodies (M. Zanetti and J.D. Capra, eds., Harwood Academic Publishers, 1995).

Definitions

[0050] An "antibody" is an immunoglobulin molecule capable of specific binding to a target, such as a carbohydrate, polynucleotide, lipid, polypeptide, etc., through at least one antigen recognition site, located in the variable region of the immunoglobulin molecule. As used herein, the term encompasses not only intact polyclonal or monoclonal antibodies, but also fragments thereof (such as Fab, Fab', $F(ab')_2$, Fv), single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (such as domain antibodies), and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site. An antibody includes an antibody of any class, such as IgG, IgA, or IgM (or subclass thereof), and the antibody need not be of any particular class. Depending on the antibody amino acid sequence of the constant domain of its heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0051] As used herein, "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler and Milstein, 1975, Nature, 256:495, or may be made by recombinant DNA methods such as described in U.S. Pat. No. 4,816,567. The monoclonal antibodies may also be isolated from phage libraries generated using the techniques described in McCafferty et al., 1990, Nature, 348:552-554, for example.

[0052] As used herein, "humanized" antibodies refer to forms of non-human (e.g. murine) antibodies that are specific chimeric immunoglobulins, immunoglobulin chains, or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and biological activity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues.

Furthermore, the humanized antibody may comprise residues that are found neither in the recipient antibody nor in the imported CDR or framework sequences, but are included to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region or domain (Fc), typically that of a human immunoglobulin. Antibodies may have Fc regions modified as described in WO 99/58572. Other forms of humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody.

[0053] As used herein, "human antibody" means an antibody having an amino acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies known in the art or disclosed herein. This definition of a human antibody includes antibodies comprising at least one human heavy chain polypeptide or at least one human light chain polypeptide. One such example is an antibody comprising murine light chain and human heavy chain polypeptides. Human antibodies can be produced using various techniques known in the art. In one embodiment, the human antibody is selected from a phage library, where that phage library expresses human antibodies (Vaughan et al., 1996, Nature Biotechnology, 14:309-314; Sheets et al., 1998, PNAS, (USA) 95:6157-6162; Hoogenboom and Winter, 1991, J. Mol. Biol., 227:381; Marks et al., 1991, J. Mol. Biol., 222:581). Human antibodies can also be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. This approach is described in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016. Alternatively, the human antibody may be prepared by immortalizing human B lymphocytes that produce an antibody directed against a target antigen (such B lymphocytes may be recovered from an individual or may have been immunized in vitro). See, e.g., Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., 1991, J. Immunol., 147 (1):86-95; and U.S. Patent No. 5,750,373.

[0054] As used herein, the term "calcitonin gene-related peptide" and "CGRP" refers to any form of calcitonin gene-related peptide and variants thereof that retain at least part of the activity of CGRP. For example, CGRP may be α-CGRP or β-CGRP. As used herein, CGRP includes all mammalian species of native sequence CGRP, e.g., human, canine, feline, equine, and bovine.

[0055] As used herein, an "anti-CGRP antagonist antibody" (interchangeably termed "anti-CGRP antibody") refers to an antibody that is able to bind to CGRP and inhibit CGRP biological activity and/or downstream pathway(s) mediated by CGRP signaling. An anti-CGRP antagonist antibody encompasses antibodies that block, antagonize, suppress or reduce (including significantly) CGRP biological activity, including downstream pathways mediated by CGRP

signaling, such as receptor binding and/or elicitation of a cellular response to CGRP. For purpose of the present invention, it will be explicitly understood that the term "anti-CGRP antagonist antibody" encompasses all the previously identified terms, titles, and functional states and characteristics whereby the CGRP itself, an CGRP biological activity (including but not limited to its ability to mediate any aspect of headache), or the consequences of the biological activity, are substantially nullified, decreased, or neutralized in any meaningful degree. In some embodiment, an anti-CGRP antagonist antibody binds CGRP and prevents CGRP binding to a CGRP receptor. In other embodiments, an anti-CGRP antibody binds CGRP and prevents activation of a CGRP receptor. Examples of anti-CGRP antagonist antibodies are provided herein.

[0056] As used herein, the terms "G1" and "antibody G1" are used interchangeably to refer to an antibody produced by expression vectors having deposit numbers of ATCC PTA-6867 and ATCC PTA-6866. The amino acid sequence of the heavy chain and light chain variable regions are shown in Figure 5. The CDR portions of antibody G1 (including Chothia and Kabat CDRs) are diagrammatically depicted in Figure 5. The polynucleotides encoding the heavy and light chain variable regions are shown in SEQ ID NO:9 and SEQ ID NO:10. The characterization of G1 is described in the Examples.

[0057] The terms "polypeptide", "oligopeptide", "peptide" and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

[0058] "Polynucleotide," or "nucleic acid," as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. Other types of modifications include, for example, "caps", substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (e.g., nucleases, toxins, antibodies, signal peptides, ply-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing

chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid supports. The 5' and 3' terminal OH can be phosphorylated or substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, α-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs and abasic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S("thioate"), P(S)S ("dithioate"), $(O)NR_2$ ("amidate"), P(O)R, P(O)OR', CO or CH_2 ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

[0059] A "variable region" of an antibody refers to the variable region of the antibody light chain or the variable region of the antibody heavy chain, either alone or in combination. The variable regions of the heavy and light chain each consist of four framework regions (FR) connected by three complementarity determining regions (CDRs) also known as hypervariable regions. The CDRs in each chain are held together in close proximity by the FRs and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies. There are at least two techniques for determining CDRs: (1) an approach based on cross-species sequence variability (i.e., Kabat et al. Sequences of Proteins of Immunological Interest, (5th ed., 1991, National Institutes of Health, Bethesda MD)); and (2) an approach based on crystallographic studies of antigen-antibody complexes (Al-lazikani et al (1997) J. Molec. Biol. 273:927-948)). As used herein, a CDR may refer to CDRs defined by either approach or by a combination of both approaches.

[0060] A "constant region" of an antibody refers to the constant region of the antibody light chain or the constant region of the antibody heavy chain, either alone or in combination.

[0061] An epitope that "preferentially binds" or "specifically binds" (used interchangeably herein) to an antibody or a polypeptide is a term well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit "specific binding" or "preferential binding" if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. An antibody "specifically binds" or "preferentially binds" to a target if it binds with greater affinity, avidity, more readily, and/or

with greater duration than it binds to other substances. For example, an antibody that specifically or preferentially binds to a CGRP epitope is an antibody that binds this epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other CGRP epitopes or non-CGRP epitopes. It is also understood by reading this definition that, for example, an antibody (or moiety or epitope) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, "specific binding" or "preferential binding" does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means preferential binding.

[0062] As used herein, "substantially pure" refers to material which is at least 50% pure (i.e., free from contaminants), more preferably at least 90 % pure, more preferably at least 95% pure, more preferably at least 98% pure, more preferably at least 99% pure.

[0063] A "host cell" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected in vivo with a polynucleotide(s)disclosed herein.

[0064] The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The numbering of the residues in the Fc region is that of the EU index as in Kabat. Kabat et al., Sequences of Proteins of Imunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3.

[0065] As used herein, "Fc receptor" and "FcR" describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors. FcyRII receptors include FcyRIIA (an "activating receptor") and FcyRIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. FcRs are reviewed in Ravetch and Kinet, 1991, Ann. Rev. Immunol., 9:457-92; Capel et al., 1994, Immunomethods, 4:25-34; and de Haas et al., 1995, J. Lab. Clin. Med., 126:330-41. "FcR" also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., 1976, J. Immunol., 117:587; and Kim et al., 1994, J. Immunol., 24:249).

[0066] "Complement dependent cytotoxicity" and "CDC" refer to the lysing of a target in the

presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g. an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods, 202:163 (1996), may be performed.

[0067] A "functional Fc region" possesses at least one effector function of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down-regulation of cell surface receptors (e.g. B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (e.g. an antibody variable domain) and can be assessed using various assays known in the art for evaluating such antibody effector functions.

[0068] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, yet retains at least one effector function of the native sequence Fc region. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, e.g. from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% sequence identity with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% sequence identity therewith, more preferably at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity therewith.

[0069] As used herein "antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which nonspecific cytotoxic cells that express Fc receptors (FcRs) (e.g. natural killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. ADCC activity of a molecule of interest can be assessed using an in vitro ADCC assay, such as that described in U.S. Patent No. 5,500,362 or 5,821,337. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al., 1998, PNAS (USA), 95:652-656.

[0070] As used herein, "treatment" is an approach for obtaining beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: improvement in any aspect of a headache including lessening severity, alleviation of pain intensity, and other associated symptoms, reducing frequency of recurrence, increasing the quality of life of those suffering from the headache, and decreasing dose of other medications required to treat the headache.. For cluster headache, other associated symptoms include, but are not limited to swelling under or around

the eyes, excessive tears, red eye, Rhinorrhea or nasal congestion, and red flushed face.

[0071] "Reducing incidence" of headache means any of reducing severity (which can include reducing need for and/or amount of (e.g., exposure to) other drugs and/or therapies generally used for this condition, including, for example, ergotamine, dihydroergotamine, or triptans for migraine), duration, and/or frequency (including, for example, delaying or increasing time to next episodic attack in an individual). As is understood by those skilled in the art, individuals may vary in terms of their response to treatment, and, as such, for example, a "method of reducing incidence of headache in an individual" reflects administering the anti-CGRP antagonist antibody based on a reasonable expectation that such administration may likely cause such a reduction in incidence in that particular individual.

[0072] "Ameliorating" headache or one or more symptoms of headache means a lessening or improvement of one or more symptoms of headache as compared to not administering an anti-CGRP antagonist antibody. "Ameliorating" also includes shortening or reduction in duration of a symptom.

[0073] As used herein, "controlling headache" refers to maintaining or reducing severity or duration of one or more symptoms of headache or frequency of headache attacks in an individual (as compared to the level before treatment). For example, the duration or severity of head pain, or frequency of attacks is reduced by at least about any of 10%, 20%, 30%, 40%, 50%, 60%, or 70% in the individual as compared to the level before treatment.

[0074] As used therein, "delaying" the development of headache means to defer, hinder, slow, retard, stabilize, and/or postpone progression of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individuals being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop headache. A method that "delays" development of the symptom is a method that reduces probability of developing the symptom in a given time frame and/or reduces extent of the symptoms in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of subjects.

[0075] "Development" or "progression" of headache means initial manifestations and/or ensuing progression of the disorder. Development of headache can be detectable and assessed using standard clinical techniques as well known in the art. However, development also refers to progression that may be undetectable. For purpose of this invention, development or progression refers to the biological course of the symptoms. "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of headache includes initial onset and/or recurrence.

[0076] As used herein, an "effective dosage" or "effective amount" of drug, compound, or pharmaceutical composition is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing

the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. For therapeutic use, beneficial or desired results include clinical results such as reducing pain intensity, duration, or frequency of headache attack, and decreasing one or more symptoms resulting from headache (biochemical, histological and/or behavioral), including its complications and intermediate pathological phenotypes presenting during development of the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication, and/or delaying the progression of the disease of patients. An effective dosage can be administered in one or more administrations. For purposes of this invention, an effective dosage of drug, compound, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of a drug, compound, or pharmaceutical composition may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

[0077] An "individual" or a "subject" is a mammal, more preferably a human. Mammals also include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, mice and rats.

[0078] As used herein, "vector" means a construct, which is capable of delivering, and preferably expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, DNA or RNA expression vectors encapsulated in liposomes, and certain eukaryotic cells, such as producer cells.

[0079] As used herein, "expression control sequence" means a nucleic acid sequence that directs transcription of a nucleic acid. An expression control sequence can be a promoter, such as a constitutive or an inducible promoter, or an enhancer. The expression control sequence is operably linked to the nucleic acid sequence to be transcribed.

[0080] As used herein, "pharmaceutically acceptable carrier" or "pharmaceutical acceptable excipient" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Preferred diluents for aerosol or parenteral administration are phosphate buffered saline or normal (0.9%) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, Remington's Pharmaceutical Sciences, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, PA,

1990; and Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing, 2000).

[0081] The term "k_{on}", as used herein, is intended to refer to the rate constant for association of an antibody to an antigen.

[0082] The term "k_{off}", as used herein, is intended to refer to the rate constant for dissociation of an antibody from the antibody/antigen complex.

[0083] The term "K_D", as used herein, is intended to refer to the equilibrium dissociation constant of an antibody-antigen interaction.

[0084] As used herein, the term "vasomotor symptom," is intended to refer to conditions related to vasodilation and include cluster headache.

A. Methods for preventing or treating vasomotor symptoms

[0085] In one aspect, the invention provides an anti-CGRP antagonist antibody for use in a method for treating or preventing cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody.

[0086] In another aspect, the invention provides an anti-CGRP antagonist antibody for use in a method for ameliorating, controlling, reducing incidence of, or delaying the development or progression of cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody.

[0087] In another aspect, the invention provides an anti-CGRP antagonist antibody for use in methods for ameliorating, controlling, reducing incidence of, or delaying the development or progression of cluster headache in an individual comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody in combination with at least one additional agent useful for treating headache.

[0088] Such additional agents include, but are not limited to, 5-HT agonists and NSAIDs. For example, the antibody and the at least one additional agent can be concomitantly administered, i.e., they can be given in close enough temporal proximity to allow their individual therapeutic effects to overlap. For example, the amount of 5-HT agonist or NSAID administered in combination with an anti-CGRP antibody should be sufficient to reduce the frequency of headache relapse in patients or produce longer lasting efficacy compared to the administration of either one of these agents in the absence of the other. This procedure may be used to treat headaches falling into any of a wide variety of classes including: migraine with or without aura; hemiplegic migraine; cluster headaches; migrainous neuralgia; chronic headaches; tension headaches; headaches resulting from other medical conditions (such as

infection or increased pressure in the skull due to a tumor); chronic paroxysmal hemicrania; miscellaneous headache unassociated with a structural lesion; headache associated with a non-vascular intracranial disorder; headache associated with the administration of a substance or its withdrawal; headache associated with noncephalic infection; headache associated with a metabolic disorder; headache associated with a disorder of the cranium, neck, eyes, ears, nose, sinuses, teeth, mouth or other facial or cranial structure; cranial neuralgias; and nerve trunk pain and deafferentiation pain.

[0089] Those skilled in the art will be able to determine appropriate dosage amounts for particular agents to be used in combination with an anti-CGRP antibody. For example, sumatriptan may be administered in a dosage from about 0.01 to about 300 mg. When administered non-parenterally, the typical dosage of sumatriptan is from about 25 to about 100 mg with about 50 mg being generally preferred and, when administered parenterally, the preferred dosage is about 6 mg. However, these dosages may be varied according to methods standard in the art so that they are optimized for a particular patient or for a particular combination therapy. Further, for example, celecoxib may be administered in an amount of between 50 and 500 mg.
Reference to anti-CGRP antagonist antibodies also include compositions comprising one or more of these agents. These compositions may further comprise suitable excipients, such as pharmaceutically acceptable excipients including buffers, which are well known in the art. The anti-CGRP antagonist antibodies for use in the present invention can be used alone or in combination with other conventional methods of treatment.

[0090] The anti-CGRP antagonist antibody can be administered to an individual via any suitable route. It should be apparent to a person skilled in the art that the examples described herein are not intended to be limiting but to be illustrative of the techniques available. Accordingly, in some embodiments, the anti-CGRP antagonist antibody is administered to a individual in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, sublingually, intrasynovial, via insufflation, intrathecal, oral, inhalation or topical routes. Administration can be systemic, e.g., intravenous administration, or localized. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, anti-CGRP antagonist antibody can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder.

[0091] In one embodiment, an anti-CGRP antagonist antibody is administered via site-specific or targeted local delivery techniques. Examples of site-specific or targeted local delivery techniques include various implantable depot sources of the anti-CGRP antagonist antibody or local delivery catheters, such as infusion catheters, an indwelling catheter, or a needle catheter, synthetic grafts, adventitial wraps, shunts and stents or other implantable devices, site specific carriers, direct injection, or direct application. See, e.g., PCT Publication No. WO 00/53211 and U.S. Patent No. 5,981,568.

[0092] Various formulations of an anti-CGRP antagonist antibody may be used for administration. In some embodiments, the anti-CGRP antagonist antibody may be administered neat. In some embodiments, anti-CGRP antagonist antibody and a pharmaceutically acceptable excipient may be in various formulations. Pharmaceutically acceptable excipients are known in the art, and are relatively inert substances that facilitate administration of a pharmacologically effective substance. For example, an excipient can give form or consistency, or act as a diluent. Suitable excipients include but are not limited to stabilizing agents, wetting and emulsifying agents, salts for varying osmolarity, encapsulating agents, buffers, and skin penetration enhancers. Excipients as well as formulations for parenteral and nonparenteral drug delivery are set forth in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

[0093] In some embodiments, these agents are formulated for administration by injection (e.g., intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.). Accordingly, these agents can be combined with pharmaceutically acceptable vehicles such as saline, Ringer's solution, dextrose solution, and the like. The particular dosage regimen, i.e., dose, timing and repetition, will depend on the particular individual and that individual's medical history.

[0094] An anti-CGRP antibody can be administered using any suitable method, including by injection (e.g., intraperitoneally, intravenously, subcutaneously, intramuscularly, etc.). Anti-CGRP antibodies can also be administered via inhalation, as described herein. Generally, for administration of anti-CGRP antibodies, an initial candidate dosage can be about 2 mg/kg. For the purpose of the present invention, a typical daily dosage might range from about any of 3µg/kg to 30 µg/kg to 300 µg/kg to 3 mg/kg, to 30 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. For example, dosage of about 1 mg/kg, about 2.5 mg/kg, about 5 mg/kg, about 10 mg/kg, and about 25 mg/kg may be used. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of symptoms occurs or until sufficient therapeutic levels are achieved, for example, to reduce pain. An exemplary dosing regimen comprises administering an initial dose of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg of the anti-CGRP antibody, or followed by a maintenance dose of about 1 mg/kg every other week. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. For example, in some embodiments, dosing from one-four times a week is contemplated. The progress of this therapy is easily monitored by conventional techniques and assays. The dosing regimen (including the CGRP antagonist(s) used) can vary over time.

[0095] For the purpose of the present invention, the appropriate dosage of an anti-CGRP antagonist antibody will depend on the anti-CGRP antagonist antibody (or compositions thereof) employed, the type and severity of cluster headache to be treated, whether the agent is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the agent, and the discretion of the attending physician. Typically the clinician will administer an anti-CGRP antagonist antibody, until a dosage is reached that

achieves the desired result. Dose and/or frequency can vary over course of treatment.

[0096] Empirical considerations, such as the half-life, generally will contribute to the determination of the dosage. For example, antibodies that are compatible with the human immune system, such as humanized antibodies or fully human antibodies, may be used to prolong half-life of the antibody and to prevent the antibody being attacked by the host's immune system. Frequency of administration may be determined and adjusted over the course of therapy, and is generally, but not necessarily, based on treatment and/or suppression and/or amelioration and/or delay of headache (e.g., migraine). Alternatively, sustained continuous release formulations of anti-CGRP antagonist antibodies may be appropriate. Various formulations and devices for achieving sustained release are known in the art.

[0097] In one embodiment, dosages for an anti-CGRP antagonist antibody may be determined empirically in individuals who have been given one or more administration(s) of an anti-CGRP antagonist antibody. Individuals are given incremental dosages of an anti-CGRP antagonist antibody. To assess efficacy of an anti-CGRP antagonist antibody, an indicator of the disease can be followed.

[0098] Administration of an anti-CGRP antagonist antibody in accordance with the use of the present invention can be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an anti-CGRP antagonist antibody may be essentially continuous over a preselected period of time or may be in a series of spaced dose, e.g., either before, during, or after developing cluster headache before; during; before and after; during and after; before and during; or before, during, and after developing cluster headache. Administration can be before, during and/or after any event likely to give rise to cluster headache.

[0099] In some embodiments, more than one anti-CGRP antagonist antibody may be present. At least one, at least two, at least three, at least four, at least five different, or more anti-CGRP antagonist antibody can be present. Generally, those anti-CGRP antagonist antibodies may have complementary activities that do not adversely affect each other. An antagonist anti-CGRP antibody can also be used in conjunction with other CGRP antagonists or CGRP receptor antagonists. For example, one or more of the following CGRP antagonists may be used: an anti-sense molecule directed to an CGRP (including an anti-sense molecule directed to a nucleic acid encoding CGRP), an CGRP inhibitory compound, an CGRP structural analog, a dominant-negative mutation of a CGRP receptor that binds an CGRP, and an anti-CGRP receptor antibody. An anti-CGRP antagonist antibody can also be used in conjunction with other agents that serve to enhance and/or complement the effectiveness of the agents.

[0100] Therapeutic formulations of the anti-CGRP antagonist antibody used in accordance with the use of the present invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing

(2000)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and may comprise buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosacchandes, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0101] Liposomes containing the anti-CGRP antagonist antibody are prepared by methods known in the art, such as described in Epstein, et al., Proc. Natl. Acad. Sci. USA 82:3688 (1985); Hwang, et al., Proc. Natl Acad. Sci. USA 77:4030 (1980); and U.S. Pat. Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in U.S. Patent No. 5,013,556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter.

[0102] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington, The Science and Practice of Pharmacy 20th Ed. Mack Publishing (2000).

[0103] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or 'poly(v nylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT ™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[0104] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes. Therapeutic anti-

CGRP antagonist antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0105] Diagnosis or assessment of headache is well-established in the art. Assessment may be performed based on subjective measures, such as patient characterization of symptoms. For example, migraine may be diagnosed based on the following criteria: 1) episodic attacks of headache lasting 4 to 72 hours; 2) with two of the following symptoms: unilateral pain, throbbing, aggravation on movement, and pain of moderate or severe intensity; and 3) one of the following symptoms: nausea or vomiting, and photophobia or phonophobia. Goadsby et al., N. Engl. J. Med. 346:257-270, 2002.

[0106] Treatment efficacy can be assessed by methods well-known in the art. For example, pain relief may be assessed. Accordingly, in some embodiments, pain relief is subjectively observed after 1, 2, or a few hours after administering an anti-CGRP antibody. In some embodiments, frequency of headache attacks is subjectively observed after administering an anti-CGRP antibody.

B. Anti-CGRP antagonist antibodies

[0107] The invention uses an anti-CGRP antagonist antibody, which refers to any antibody molecule that blocks, suppresses or reduces (including significantly) CGRP biological activity, including downstream pathways mediated by CGRP signaling, such as receptor binding and/or elicitation of a cellular response to CGRP.

[0108] An anti-CGRP antagonist antibody should exhibit any one or more of the following characteristics: (a) bind to CGRP; (b) block CGRP from binding to its receptor(s); (c) block or decrease CGRP receptor activation (including cAMP activation); (d) inhibit CGRP biological activity or downstream pathways mediated by CGRP signaling function; (e) prevent, ameliorate, or treat any aspect of headache (e.g., migraine); (f) increase clearance of CGRP; and (g) inhibit (reduce) CGRP synthesis, production or release. Anti-CGRP antagonist antibodies are known in the art. See, e.g., Tan et al., Clin. Sci. (Lond). 89:565-73, 1995; Sigma (Missouri, US), product number C7113 (clone #4901); Plourde et al., Peptides 14:1225-1229, 1993.

[0109] For purposes of this invention, the antibody reacts with CGRP in a manner that inhibits CGRP and/or downstream pathways mediated by the CGRP signaling function. In some embodiments, the anti-CGRP antagonist antibody recognizes human CGRP. In some embodiments, the anti-CGRP antagonist antibody binds to both human α-CGRP and β-CGRP. In some embodiments, the anti-CGRP antagonist antibody binds human and rat CGRP. In some embodiments, the anti-CGRP antagonist antibody binds the C-terminal fragment having amino acids 25-37 of CGRP. In some embodiments, the anti-CGRP antagonist antibody binds a C-terminal epitope within amino acids 25-37 of CGRP.

[0110] The antibodies useful in the present invention can encompass monoclonal antibodies, polyclonal antibodies, antibody fragments (e.g., Fab, Fab', F(ab')2, Fv, Fc, etc.), chimeric antibodies, bispecific antibodies, heteroconjugate antibodies, single chain (ScFv), mutants thereof, fusion proteins comprising an antibody portion (e.g., a domain antibody), humanized antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The antibodies may be murine, rat, human, or any other origin (including chimeric or humanized antibodies).

[0111] In some embodiments, the anti-CGRP antagonist antibody is a monoclonal antibody. In some embodiments, the anti-CGRP antagonist antibody is humanized. In some embodiments, the antibody is human. In some embodiments, the anti-CGRP antagonist antibody is antibody G1 (as described herein). In some embodiments, the anti-CGRP antagonist antibody comprises one or more CDR(s) (such as one, two, three, four, five, or, in some embodiments, all six CDRs) of antibody G1 or variants of G1 shown in Table 6. In still other embodiments, the anti-CGRP antagonist antibody comprises the amino acid sequence of the heavy chain variable region shown in Figure 5 (SEQ ID NO:1) and the amino acid sequence of the light chain variable region shown in Figure 5 (SEQ ID NO:2).

[0112] In some embodiments, the antibody comprises a modified constant region, such as a constant region that is immunologically inert described herein. In some embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624;and WO 99/58572. In other embodiments, the antibody comprises a human heavy chain IgG2 constant region comprising the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence). Eur. J. Immunol. (1999) 29:2613-2624. In some embodiments, the antibody comprises a constant region of IgG4 comprising the following mutations: E233F234L235 to P233V234A235. In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the oligosaccharide attachment residue (such as Asn297) and/or flanking residues that are part of the N-glycosylation recognition sequence in the constant region . In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked glycosylation enzymatically or by expression in a glycosylation deficient host cell.

[0113] The binding affinity (K_D) of an anti-CGRP antagonist antibody to CGRP (such as human α-CGRP) can be about 0.02 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, or about 2 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM.

[0114] One way of determining binding affinity of antibodies to CGRP is by measuring binding affinity of monofunctional Fab fragments of the antibody. To obtain monofunctional Fab fragments, an antibody (for example, IgG) can be cleaved with papain or expressed recombinantly. The affinity of an anti-CGRP Fab fragment of an antibody can be determined by surface plasmon resonance (Biacore3000™ surface plasmon resonance (SPR) system, Biacore, INC, Piscataway NJ) equipped with pre-immobilized streptavidin sensor chips (SA) using HBS-EP running buffer (0.01M HEPES, pH 7,4, 0.15 Nacl, 3mM EDTA, 0.005% v/v Surfactant P20). Biotinylated human CGRP (or any other CGRP) can be diluted into HBS-EP buffer to a concentration of less than 0.5 ug/mL and injected across the individual chip channels using variable contact times, to achieve two ranges of antigen density, either 50-200 response units (RU) for detailed kinetic studies or 800-1,000 RU for screening assays. Regeneration studies have shown that 25mM NaOH in 25% v/v ethanol effectively removes the bound Fab while keeping the activity of CGRP on the chip for over 200 injections. Typically, serial dilutions (spanning concentrations of 0.1-10x estimated K_D) of purified Fab samples are injected for 1 min at 100 µL/minute and dissociation times of up to 2 hours are allowed. The concentrations of the Fab proteins are determined by ELISA and/or SDS-PAGE electrophoresis using a Fab of known concentration (as determined by amino acid analysis) as a standard. Kinetic association rates (k_{on}) and dissociation rates (k_{off}) are obtained simultaneously by fitting the data globally to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Equilibrium dissociation constant (K_D) values are calculated as k_{off}/k_{on}. This protocol is suitable for use in determining binding affinity of an antibody to any CGRP, including human CGRP, CGRP of another mammalian (such as mouse CGRP, rat CGRP, primate CGRP), as well as different forms of CGRP (such as α and β form). Binding affinity of an antibody is generally measured at 25°C, but can also be measured at 37°C.

[0115] The anti-CGRP antagonist antibodies may be made by any method known in the art. The route and schedule of immunization of the host animal are generally in keeping with established and conventional techniques for antibody stimulation and production, as further described herein. General techniques for production of human and mouse antibodies are known in the art and are described herein.

[0116] It is contemplated that any mammalian subject including humans or antibody producing cells therefrom can be manipulated to serve as the basis for production of mammalian, including human, hybridoma cell lines. Typically, the host animal is inoculated intraperitoneally, intramuscularly, orally, subcutaneously, intraplantar, and/or intradermally with an amount of immunogen, including as described herein.

[0117] Hybridomas can be prepared from the lymphocytes and immortalized myeloma cells using the general somatic cell hybridization technique of Kohler, B. and Milstein, C. (1975) Nature 256:495-497 or as modified by Buck, D. W., et al., In Vitro, 18:377-381 (1982). Available myeloma lines, including but not limited to X63-Ag8.653 and those from the Salk Institute, Cell Distribution Center, San Diego, Calif., USA, may be used in the hybridization. Generally, the technique involves fusing myeloma cells and lymphoid cells using a fusogen

such as polyethylene glycol, or by electrical means well known to those skilled in the art. After the fusion, the cells are separated from the fusion medium and grown in a selective growth medium, such as hypoxanthine-aminopterin-thymidine (HAT) medium, to eliminate unhybridized parent cells. Any of the media described herein, supplemented with or without serum, can be used for culturing hybridomas that secrete monoclonal antibodies. As another alternative to the cell fusion technique, EBV immortalized B cells may be used to produce the anti-CGRP monoclonal antibodies of the subject invention. The hybridomas are expanded and subcloned, if desired, and supernatants are assayed for anti-immunogen activity by conventional immunoassay procedures (e.g., radioimmunoassay, enzyme immunoassay, or fluorescence immunoassay).

[0118] Hybridomas that may be used as source of antibodies encompass all derivatives, progeny cells of the parent hybridomas that produce monoclonal antibodies specific for CGRP, or a portion thereof.

[0119] Hybridomas that produce such antibodies may be grown in vitro or in vivo using known procedures. The monoclonal antibodies may be isolated from the culture media or body fluids, by conventional immunoglobulin purification procedures such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography, and ultrafiltration, if desired. Undesired activity if present, can be removed, for example, by running the preparation over adsorbents made of the immunogen attached to a solid phase and eluting or releasing the desired antibodies off the immunogen. Immunization of a host animal with a human CGRP, or a fragment containing the target amino acid sequence conjugated to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaradehyde, succinic anhydride, SOCl2, or R1N=C=NR, where R and R1 are different alkyl groups, can yield a population of antibodies (e.g., monoclonal antibodies).

[0120] If desired, the anti-CGRP antagonist antibody (monoclonal or polyclonal) of interest may be sequenced and the polynucleotide sequence may then be cloned into a vector for expression or propagation. The sequence encoding the antibody of interest may be maintained in vector in a host cell and the host cell can then be expanded and frozen for future use. In an alternative, the polynucleotide sequence may be used for genetic manipulation to "humanize" the antibody or to improve the affinity, or other characteristics of the antibody. For example, the constant region may be engineered to more resemble human constant regions to avoid immune response if the antibody is used in clinical trials and treatments in humans. It may be desirable to genetically manipulate the antibody sequence to obtain greater affinity to CGRP and greater efficacy in inhibiting CGRP. It will be apparent to one of skill in the art that one or more polynucleotide changes can be made to the anti-CGRP antagonist antibody and still maintain its binding ability to CGRP.

[0121] There are four general steps to humanize a monoclonal antibody. These are: (1)

determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains (2) designing the humanized antibody, i.e., deciding which antibody framework region to use during the humanizing process (3) the actual humanizing methodologies/techniques and (4) the transfection and expression of the humanized antibody. See, for example, U.S. Patent Nos. 4,816,567; 5,807,715; 5,866,692; 6,331,415; 5,530,101; 5,693,761; 5,693,762; 5,585,089; and 6,180,370.

[0122] A number of "humanized" antibody molecules comprising an antigen-binding site derived from a non-human immunoglobulin have been described, including chimeric antibodies having rodent or modified rodent V regions and their associated complementarity determining regions (CDRs) fused to human constant domains. See, for example, Winter et al. Nature 349:293-299 (1991), Lobuglio et al. Proc. Nat. Acad. Sci. USA 86:4220-4224 (1989), Shaw et al. J Immunol. 138:4534-4538 (1987), and Brown et al. Cancer Res. 47:3577-3583 (1987). Other references describe rodent CDRs grafted into a human supporting framework region (FR) prior to fusion with an appropriate human antibody constant domain. See, for example, Riechmann et al. Nature 332:323-327 (1988), Verhoeyen et al. Science 239:1534-1536 (1988), and Jones et al. Nature 321:522-525 (1986). Another reference describes rodent CDRs supported by recombinantly veneered rodent framework regions. See, for example, European Patent Publication No. 0519596. These "humanized" molecules are designed to minimize unwanted immunological response toward rodent anti-human antibody molecules which limits the duration and effectiveness of therapeutic applications of those moieties in human recipients. For example, the antibody constant region can be engineered such that it is immunologically inert (e.g., does not trigger complement lysis). See, e.g. WO 99/58572. Other methods of humanizing antibodies that may also be utilized are disclosed by Daugherty et al., Nucl. Acids Res. 19:2471-2476 (1991) and in U.S. Patent Nos. 6,180,377; 6,054,297; 5,997,867; 5,866,692; 6,210,671; and 6,350,861; and in PCT Publication No. WO 01/27160.

[0123] In yet another alternative, fully human antibodies may be obtained by using commercially available mice that have been engineered to express specific human immunoglobulin proteins. Transgenic animals that are designed to produce a more desirable (e.g., fully human antibodies) or more robust immune response may also be used for generation of humanized or human antibodies. Examples of such technology are Xenomouse ™ from Abgenix, Inc. (Fremont, CA) and HuMAb-Mouse® and TC Mouse™ from Medarex, Inc. (Princeton, NJ).

[0124] In an alternative, antibodies may be made recombinantly and expressed using any method known in the art. In another alternative, antibodies may be made recombinantly by phage display technology. See, for example, U.S. Patent Nos. 5,565,332; 5,580,717; 5,733,743; and 6,265,150; and Winter et al., Annu. Rev. Immunol. 12:433-455 (1994). Alternatively, the phage display technology (McCafferty et al., Nature 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody

fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats; for review see, e.g., Johnson, Kevin S. and Chiswell, David J., Current Opinion in Structural Biology 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Mark et al., J. Mol. Biol. 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). In a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling." Marks, et al., Bio/Technol. 10:779-783 (1992)). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the pM-nM range. A strategy for making very large phage antibody repertoires (also known as "the mother-of-all libraries") has been described by Waterhouse et al., Nucl. Acids Res. 21:2265-2266 (1993). Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection on antigen results in isolation of human variable regions capable of restoring a functional antigen-binding site, i.e., the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT Publication No. WO 93/06213, published April 1, 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

[0125] It is apparent that although the above discussion pertains to humanized antibodies, the general principles discussed are applicable to customizing antibodies for use, for example, in dogs, cats, primate, equines and bovines. It is further apparent that one or more aspects of humanizing an antibody described herein may be combined, e.g., CDR grafting, framework mutation and CDR mutation.

[0126] Antibodies may be made recombinantly by first isolating the antibodies and antibody producing cells from host animals, obtaining the gene sequence, and using the gene sequence to express the antibody recombinantly in host cells (e.g., CHO cells). Another method which

may be employed is to express the antibody sequence in plants (e.g., tobacco) or transgenic milk. Methods for expressing antibodies recombinantly in plants or milk have been disclosed. See, for example, Peeters, et al. Vaccine 19:2756 (2001); Lonberg, N. and D. Huszar Int.Rev.Immunol 13:65 (1995); and Pollock, et al., J Immunol Methods 231:147(1999). Methods for making derivatives of antibodies, e.g., humanized, single chain, etc. are known in the art.

[0127] Immunoassays and flow cytometry sorting techniques such as fluorescence activated cell sorting (FACS) can also be employed to isolate antibodies that are specific for CGRP.

[0128] The antibodies can be bound to many different carriers. Carriers can be active and/or inert. Examples of well-known carriers include polypropylene, polystyrene, polyethylene, dextran, nylon, amylases, glass, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation. In some embodiments, the carrier comprises a moiety that targets the myocardium.

[0129] DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the monoclonal antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors (such as expression vectors disclosed in PCT Publication No. WO 87/04462), which are then transfected into host cells such as E. coli cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. See, e.g., PCT Publication No. WO 87/04462. The DNA also may be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences, Morrison et al., Proc. Nat. Acad. Sci. 81:6851 (1984), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-CGRP monoclonal antibody herein.

[0130] Anti-CGRP antagonist antibodies can be identified or characterized using methods known in the art, whereby reduction, amelioration, or neutralization of an CGRP biological activity is detected and/or measured. For example, anti-CGRP antagonist antibody can also be identified by incubating a candidate agent with CGRP and monitoring any one or more of the following characteristics: (a) bind to CGRP; (b) block CGRP from binding to its receptor(s); (c) block or decrease CGRP receptor activation (including cAMP activation); (d) inhibit CGRP biological activity or downstream pathways mediated by CGRP signaling function; (e) prevent, ameliorate, or treat any aspect of headache (e.g., migraine); (f) increase clearance of CGRP; and (g) inhibit (reduce) CGRP synthesis, production or release. In some embodiments, an anti-CGRP antagonist antibody is identified by incubating a candidate agent with CGRP and

monitoring binding and/or attendant reduction or neutralization of a biological activity of CGRP. The binding assay may be performed with purified CGRP polypeptide(s), or with cells naturally expressing, or transfected to express, CGRP polypeptide(s). In one embodiment, the binding assay is a competitive binding assay, where the ability of a candidate antibody to compete with a known anti-CGRP antagonist for CGRP binding is evaluated. The assay may be performed in various formats, including the ELISA format. In other embodiments, an anti-CGRP antagonist antibody is identified by incubating a candidate agent with CGRP and monitoring binding and attendant inhibition of CGRP receptor activation expressed on the surface of a cell.

[0131] Following initial identification, the activity of a candidate anti-CGRP antagonist antibody can be further confirmed and refined by bioassays, known to test the targeted biological activities. Alternatively, bioassays can be used to screen candidates directly. For example, CGRP promotes a number of measurable changes in responsive cells. These include, but are not limited to, stimulation of cAMP in the cell (e.g., SK-N-MC cells). Antagonist activity may also be measured using animal models, such as measuring skin vasodilatation induced by stimulation of the rat saphenous nerve. Escott et al., Br. J. Pharmacol. 110: 772-776, 1993. Animal models of headaches (such as, migraine) may further be used for testing efficacy of antagonist antibodies or polypeptides. Reuter, et al., Functional Neurology (15) Suppl.3, 2000. Some of the methods for identifying and characterizing anti-CGRP antagonist antibody are described in detail in the Examples.

[0132] Anti-CGRP antagonist antibodies may be characterized using methods well known in the art. For example, one method is to identify the epitope to which it binds, or "epitope mapping." There are many methods known in the art for mapping and characterizing the location of epitopes on proteins, including solving the crystal structure of an antibody-antigen complex, competition assays, gene fragment expression assays, and synthetic peptide-based assays, as described, for example, in Chapter 11 of Harlow and Lane, Using Antibodies, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999. In an additional example, epitope mapping can be used to determine the sequence to which an anti-CGRP antagonist antibody binds. Epitope mapping is commercially available from various sources, for example, Pepscan Systems (Edelhertweg 15, 8219 PH Lelystad, The Netherlands). The epitope can be a linear epitope, i.e., contained in a single stretch of amino acids, or a conformational epitope formed by a three-dimensional interaction of amino acids that may not necessarily be contained in a single stretch. Peptides of varying lengths (e.g., at least 4-6 amino acids long) can be isolated or synthesized (e.g., recombinantly) and used for binding assays with an anti-CGRP antagonist antibody. In another example, the epitope to which the anti-CGRP antagonist antibody binds can be determined in a systematic screening by using overlapping peptides derived from the CGRP sequence and determining binding by the anti-CGRP antagonist antibody. According to the gene fragment expression assays, the open reading frame encoding CGRP is fragmented either randomly or by specific genetic constructions and the reactivity of the expressed fragments of CGRP with the antibody to be tested is determined. The gene fragments may, for example, be produced by PCR and then transcribed and translated into protein in vitro, in the presence of radioactive amino acids. The binding of the antibody to the radioactively labeled CGRP fragments is then determined by

immunoprecipitation and gel electrophoresis. Certain epitopes can also be identified by using large libraries of random peptide sequences displayed on the surface of phage particles (phage libraries). Alternatively, a defined library of overlapping peptide fragments can be tested for binding to the test antibody in simple binding assays. In an additional example, mutagenesis of an antigen binding domain, domain swapping experiments and alanine scanning mutagenesis can be performed to identify residues required, sufficient, and/or necessary for epitope binding. For example, domain swapping experiments can be performed using a mutant CGRP in which various fragments of the CGRP polypeptide have been replaced (swapped) with sequences from a closely related, but antigenically distinct protein (such as another member of the neurotrophin protein family). By assessing binding of the antibody to the mutant CGRP, the importance of the particular CGRP fragment to antibody binding can be assessed.

[0133] Yet another method which can be used to characterize an anti-CGRP antagonist antibody is to use competition assays with other antibodies known to bind to the same antigen, i.e., various fragments on CGRP, to determine if the anti-CGRP antagonist antibody binds to the same epitope as other antibodies. Competition assays are well known to those of skill in the art.

C. Antibody G1 and related antibodies

[0134] This invention encompasses the use of antibody G1 and its variants shown in Table 6.

[0135] The anti-CGRP antagonist antibodies are characterized by any (one or more) of the following characteristics: (a) bind to CGRP; (b) block CGRP from binding to its receptor(s); (c) block or decrease CGRP receptor activation (including cAMP activation); (d) inhibit CGRP biological activity or downstream pathways mediated by CGRP signaling function; (e) prevent, ameliorate, or treat any aspect of headache (e.g., migraine); (f) increase clearance of CGRP; and (g) inhibit (reduce) CGRP synthesis, production or release.

[0136] Any of the following can be used in the invention (a) antibody G1 or its variants shown in Table 6; (b) a fragment or a region of antibody G1 or its variants shown in Table 6; (c) a light chain of antibody G1 or its variants shown in Table 6; (d) a heavy chain of antibody G1 or its variants shown in Table 6; (e) one or more variable region(s) from a light chain and/or a heavy chain of antibody G1 or its variants shown in Table 6; (f) one or more CDR(s) (one, two, three, four, five or six CDRs) of antibody G1 or its variants shown in Table 6; (g) CDR H3 from the heavy chain of antibody G1; (h) CDR L3 from the light chain of antibody G1 or its variants shown in Table 6; (i) three CDRs from the light chain of antibody G1 or its variants shown in Table 6; (j) three CDRs from the heavy chain of antibody G1 or its variants shown in Table 6; (k) three CDRs from the light chain and three CDRs from the heavy chain, of antibody G1 or its variants shown in Table 6; and (l) an antibody comprising any one of (b) through (k).

[0137] The CDR portions of antibody G1 (including Chothia and Kabat CDRs) are

diagrammatically depicted in Figure 5. Determination of CDR regions is well within the skill of the art. It is understood that in some embodiments, CDRs can be a combination of the Kabat and Chothia CDR (also termed "combined CDRs" or "extended CDRs"). In some embodiments, the CDRs are the Kabat CDRs. In other embodiments, the CDRs are the Chothia CDRs. In other words, in embodiments with more than one CDR, the CDRs may be any of Kabat, Chothia, combination CDRs, or combinations thereof.

[0138] In some embodiments, an antibody useful in the invention comprises at least one CDR, at least two, at least three, or at least four, at least five, or all six CDRs that are substantially identical to at least one CDR, at least two, at least three, at least four, at least five or all six CDRs of G1 or its variants shown in Table 6. Other embodiments include antibodies which have at least two, three, four, five, or six CDR(s) that are substantially identical to at least two, three, four, five or six CDRs of G1 or derived from G1. In some embodiments, the at least one, two, three, four, five, or six CDR(s) are at least about 85%, 86%, 87%, 88%, 89%, 90%, 95%, 96%, 97%, 98%, or 99% identical to at least one, two, three, four, five or six CDRs of G1 or its variants shown in Table 6. It is understood that, for purposes of this invention, binding specificity and/or overall activity is generally retained, although the extent of activity may vary compared to G1 or its variants shown in Table 6 (may be greater or lesser).

[0139] An antibody useful in the invention comprises an amino acid sequence of G1 or its variants shown in Table 6 that has any of the following: at least 5 contiguous amino acids, at least 8 contiguous amino acids, at least about 10 contiguous amino acids, at least about 15 contiguous amino acids, at least about 20 contiguous amino acids, at least about 25 contiguous amino acids, at least about 30 contiguous amino acids of a sequence of G1 or its variants shown in Table 6, wherein at least 3 of the amino acids are from a variable region of G1 (Figure 5) or its variants shown in Table 6. In one embodiment, the variable region is from a light chain of G1. In another embodiment, the variable region is from a heavy chain of G1. An exemplary antibody has contiguous amino acid (lengths described above) from both the heavy and light chain variable regions of G1. In another embodiment, the 5 (or more) contiguous amino acids are from a complementarity determining region (CDR) of G1 shown in Figure 5. In some embodiments, the contiguous amino acids are from a variable region of G1.

[0140] The binding affinity (K_D) of an anti-CGRP antagonist antibody to CGRP (such as human α-CGRP) can be about 0.06 to about 200 nM. In some embodiments, the binding affinity is any of about 200 nM, 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, about 60 pM, about 50 pM, about 20 pM, about 15 pM, about 10 pM, about 5 pM, or about 2 pM. In some embodiments, the binding affinity is less than any of about 250 nM, about 200 nM, about 100 nM, about 50 nM, about 10 nM, about 1 nM, about 500 pM, about 100 pM, or about 50 pM.

[0141] Single chain variable region fragments ("scFv") of antibodies such as G1 can be used in the invention. Single chain variable region fragments are made by linking light and/or heavy chain variable regions by using a short linking peptide. Bird et al. (1988) Science 242:423-426. An example of a linking peptide is (GGGGS)3 which bridges approximately 3.5 nm between the

carboxy terminus of one variable region and the amino terminus of the other variable region. Linkers of other sequences have been designed and used. Bird et al. (1988). Linkers can in turn be modified for additional functions, such as attachment of drugs or attachment to solid supports. The single chain variants can be produced either recombinantly or synthetically. For synthetic production of scFv, an automated synthesizer can be used. For recombinant production of scFv, a suitable plasmid containing polynucleotide that encodes the scFv can be introduced into a suitable host cell, either eukaryotic, such as yeast, plant, insect or mammalian cells, or prokaryotic, such as E. coli. Polynucleotides encoding the scFv of interest can be made by routine manipulations such as ligation of polynucleotides. The resultant scFv can be isolated using standard protein purification techniques known in the art.

[0142] Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993) Proc. Natl. Acad Sci. USA 90:6444-6448; Poljak, R. J., et al. (1994) Structure 2:1121-1123).

[0143] For example, bispecific antibodies, monoclonal antibodies that have binding specificities for at least two different antigens, can be prepared using the antibodies disclosed herein. Methods for making bispecific antibodies are known in the art (see, e.g., Suresh et al., 1986, Methods in Enzymology 121:210). Traditionally, the recombinant production of bispecific antibodies was based on the coexpression of two immunoglobulin heavy chain-light chain pairs, with the two heavy chains having different specificities (Millstein and Cuello, 1983, Nature 305, 537-539).

[0144] According to one approach to making bispecific antibodies, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH2 and CH3 regions. It is preferred to have the first heavy chain constant region (CH1), containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are cotransfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

[0145] In one approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric

structure, with an immunoglobulin light chain in only one half of the bispecific molecule, facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations. This approach is described in PCT Publication No. WO 94/04690, published March 3, 1994.

[0146] Heteroconjugate antibodies, comprising two covalently joined antibodies, are also within the scope of the invention. Such antibodies have been used to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (PCT application publication Nos. WO 91/00360 and WO 92/200373; EP 03089). Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents and techniques are well known in the art, and are described in U.S. Patent No. 4,676,980.

[0147] Chimeric or hybrid antibodies also may be prepared in vitro using known methods of synthetic protein chemistry, including those involving cross-linking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

[0148] Humanized antibody comprising one or more CDRs of antibody G1 or its variants shown in Table 6, or one or more CDRs derived from antibody G1 or its variants shown in Table 6 can be made using any methods known in the art. For example, four general steps may be used to humanize a monoclonal antibody.

[0149] Modifications to antibody G1 or its variants shown in Table 6, including functionally equivalent antibodies which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity can also be used. For example, the amino acid sequence of antibody G1 or its variants shown in Table 6 may be mutated to obtain an antibody with the desired binding affinity to CGRP. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Modification of polypeptides is exemplified in the Examples. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs.

[0150] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody.

[0151] Substitution variants have at least one amino acid residue in the antibody molecule

removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened.

Table 1: Amino Acid Substitutions

Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; lie
Arg (R)	Lys	Lys; Gln; Asn
Asn (N)	Gln	Gln; His; Asp, Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; lie
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

[0152] Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

1. (1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile;

2. (2) Polar without charge: Cys, Ser, Thr, Asn, Gln;
3. (3) Acidic (negatively charged): Asp, Glu;
4. (4) Basic (positively charged): Lys, Arg;
5. (5) Residues that influence chain orientation: Gly, Pro; and
6. (6) Aromatic: Trp, Tyr, Phe, His.

[0153] Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

[0154] Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

[0155] Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity. In some embodiments, no more than one to five conservative amino acid substitutions are made within a CDR domain. In other embodiments, no more than one to three conservative amino acid substitutions are made within a CDR domain. In still other embodiments, the CDR domain is CDR H3 and/or CDR L3.

[0156] Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund, 1997, Chem. Immunol. 65:111-128; Wright and Morrison, 1997, TibTECH 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., 1996, Mol. Immunol. 32:1311-1318; Wittwe and Howard, 1990, Biochem. 29:4175-4180) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hefferis and Lund, supra; Wyss and Wagner, 1996, Current Opin. Biotech. 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β(1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al., 1999, Mature Biotech. 17:176-180).

[0157] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine, asparagine-X-threonine, and asparagine-X-

cysteine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0158] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0159] The glycosylation pattern of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al., 1997, J. Biol. Chem. 272:9062-9070).

[0160] In addition to the choice of host cells, factors that affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U. S. Patent Nos. 5,047,335; 5,510,261 and 5.278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H), N-glycosidase F, endoglycosidase F1, endoglycosidase F2, endoglycosidase F3. In addition, the recombinant host cell can be genetically engineered to be defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

[0161] Other methods of modification include using coupling techniques known in the art, including, but not limited to, enzymatic means, oxidative substitution and chelation. Modifications can be used, for example, for attachment of labels for immunoassay. Modified G1 polypeptides are made using established procedures in the art and can be screened using standard assays known in the art, some of which are described below and in the Examples.

[0162] In some embodiments, the antibody comprises a modified constant region, such as a constant region that is immunologically inert or partially inert, e.g., does not trigger complement mediated lysis, does not stimulate antibody-dependent cell mediated cytotoxicity (ADCC), or does not activate microglia; or have reduced activities (compared to the unmodified antibody) in any one or more of the following: triggering complement mediated lysis, stimulating antibody-dependent cell mediated cytotoxicity (ADCC), or activating microglia. Different modifications of the constant region may be used to achieve optimal level and/or combination

of effector functions. See, for example, Morgan et al., Immunology 86:319-324 (1995); Lund et al., J. Immunology 157:4963-9 157:4963-4969 (1996); Idusogie et al., J. Immunology 164:4178-4184 (2000); Tao et al., J. Immunology 143: 2595-2601 (1989); and Jefferis et al., Immunological Reviews 163:59-76 (1998). In some embodiments, the constant region is modified as described in Eur. J. Immunol. (1999) 29:2613-2624; PCT Application No. PCT/GB99/01441; and/or UK Patent Application No. 9809951.8 In other embodiments, the antibody comprises a human heavy chain IgG2 constant region comprising the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence). Eur. J. Immunol. (1999) 29:2613-2624. In still other embodiments, the constant region is aglycosylated for N-linked glycosylation. In some embodiments, the constant region is aglycosylated for N-linked glycosylation by mutating the glycosylated amino acid residue or flanking residues that are part of the N-glycosylation recognition sequence in the constant region. For example, N-glycosylation site N297 may be mutated to A, Q, K, or H. See, Tao et al., J. Immunology 143: 2595-2601 (1989); and Jefferis et al., Immunological Reviews 163:59-76 (1998). In some embodiments, the constant region is aglycosylated for N-linked glycosylation. The constant region may be aglycosylated for N-linked glycosylation enzymatically (such as removing carbohydrate by enzyme PNGase), or by expression in a glycosylation deficient host cell.

[0163] Other antibody modifications include antibodies that have been modified as described in PCT Publication No. WO 99/58572, published November 18, 1999. These antibodies comprise, in addition to a binding domain directed at the target molecule, an effector domain having an amino acid sequence substantially homologous to all or part of a constant domain of a human immunoglobulin heavy chain. These antibodies are capable of binding the target molecule without triggering significant complement dependent lysis, or cell-mediated destruction of the target. In some embodiments, the effector domain is capable of specifically binding FcRn and/or FcγRIIb. These are typically based on chimeric domains derived from two or more human immunoglobulin heavy chain C_H2 domains. Antibodies modified in this manner are particularly suitable for use in chronic antibody therapy, to avoid inflammatory and other adverse reactions to conventional antibody therapy.

[0164] Affinity matured embodiments can also be used. For example, affinity matured antibodies can be produced by procedures known in the art (Marks et al., 1992, Bio/Technology, 10:779-783; Barbas et al., 1994, Proc Nat. Acad. Sci, USA 91:3809-3813; Schier et al., 1995, Gene, 169:147-155; Yelton et al., 1995, J. Immunol., 155:1994-2004; Jackson et al., 1995, J. Immunol., 154(7):3310-9; Hawkins et al, 1992, J. Mol. Biol., 226:889-896; and WO2004/058184).

[0165] The following methods may be used for adjusting the affinity of an antibody and for characterizing a CDR. One way of characterizing a CDR of an antibody and/or altering (such as improving) the binding affinity of a polypeptide, such as an antibody, termed "library scanning mutagenesis". Generally, library scanning mutagenesis works as follows. One or more amino acid positions in the CDR are replaced with two or more (such as 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) amino acids using art recognized methods. This

generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of two or more members (if two or more amino acids are substituted at every position). Generally, the library also includes a clone comprising the native (unsubstituted) amino acid. A small number of clones, e.g., about 20-80 clones (depending on the complexity of the library), from each library are screened for binding affinity to the target polypeptide (or other binding target), and candidates with increased, the same, decreased or no binding are identified. Methods for determining binding affinity are well-known in the art. Binding affinity may be determined using Biacore surface plasmon resonance analysis, which detects differences in binding affinity of about 2-fold or greater. Biacore is particularly useful when the starting antibody already binds with a relatively high affinity, for example a K_D of about 10 nM or lower. Screening using Biacore surface plasmon resonance is described in the Examples, herein.

[0166] Binding affinity may be determined using Kinexa Biocensor, scintillation proximity assays, ELISA, ORIGEN immunoassay (IGEN), fluorescence quenching, fluorescence transfer, and/or yeast display. Binding affinity may also be screened using a suitable bioassay.

[0167] In some embodiments, every amino acid position in a CDR is replaced (in some embodiments, one at a time) with all 20 natural amino acids using art recognized mutagenesis methods (some of which are described herein). This generates small libraries of clones (in some embodiments, one for every amino acid position that is analyzed), each with a complexity of 20 members (if all 20 amino acids are substituted at every position).

[0168] In some embodiments, the library to be screened comprises substitutions in two or more positions, which may be in the same CDR or in two or more CDRs. Thus, the library may comprise substitutions in two or more positions in one CDR. The library may comprise substitution in two or more positions in two or more CDRs. The library may comprise substitution in 3, 4, 5, or more positions, said positions found in two, three, four, five or six CDRs. The substitution may be prepared using low redundancy codons. See, e.g., Table 2 of Balint et al., (1993) Gene 137(1):109-18).

[0169] The CDR may be CDRH3 and/or CDRL3. The CDR may be one or more of CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and/or CDRH3. The CDR may be a Kabat CDR, a Chothia CDR, or an extended CDR.

[0170] Candidates with improved binding may be sequenced, thereby identifying a CDR substitution mutant which results in improved affinity (also termed an "improved" substitution). Candidates that bind may also be sequenced, thereby identifying a CDR substitution which retains binding.

[0171] Multiple rounds of screening may be conducted. For example, candidates (each comprising an amino acid substitution at one or more position of one or more CDR) with improved binding are also useful for the design of a second library containing at least the original and substituted amino acid at each improved CDR position (i.e., amino acid position in

the CDR at which a substitution mutant showed improved binding). Preparation, and screening or selection of this library is discussed further below.

[0172] Library scanning mutagenesis also provides a means for characterizing a CDR, in so far as the frequency of clones with improved binding, the same binding, decreased binding or no binding also provide information relating to the importance of each amino acid position for the stability of the antibody-antigen complex. For example, if a position of the CDR retains binding when changed to all 20 amino acids, that position is identified as a position that is unlikely to be required for antigen binding. Conversely, if a position of CDR retains binding in only a small percentage of substitutions, that position is identified as a position that is important to CDR function. Thus, the library scanning mutagenesis methods generate information regarding positions in the CDRs that can be changed to many different amino acids (including all 20 amino acids), and positions in the CDRs which cannot be changed or which can only be changed to a few amino acids.

[0173] Candidates with improved affinity may be combined in a second library, which includes the improved amino acid, the original amino acid at that position, and may further include additional substitutions at that position, depending on the complexity of the library that is desired, or permitted using the desired screening or selection method. In addition, if desired, adjacent amino acid position can be randomized to at least two or more amino acids. Randomization of adjacent amino acids may permit additional conformational flexibility in the mutant CDR, which may in turn, permit or facilitate the introduction of a larger number of improving mutations. The library may also comprise substitution at positions that did not show improved affinity in the first round of screening.

[0174] The second library is screened or selected for library members with improved and/or altered binding affinity using any method known in the art, including screening using Biacore surface plasmon resonance analysis, and selection using any method known in the art for selection, including phage display, yeast display, and ribosome display.

[0175] Also useful in the invention are fusion proteins comprising one or more fragments or regions from the antibodies (such as G1). In one embodiment, a fusion polypeptide is provided that comprises at least 10 contiguous amino acids of the variable light chain region shown in SEQ ID NO:2 (Figure 5) and/or at least 10 amino acids of the variable heavy chain region shown in SEQ ID NO:1 (Figure 5). In other embodiments, a fusion polypeptide is provided that comprises at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable light chain region shown in SEQ ID NO:2 (Figure 5) and/or at least about 10, at least about 15, at least about 20, at least about 25, or at least about 30 contiguous amino acids of the variable heavy chain region shown in SEQ ID NO:1 (Figure 5). In another embodiment, the fusion polypeptide comprises a light chain variable region and/or a heavy chain variable region of G1, as shown in SEQ ID NO:2 and SEQ ID NO:1 of Figure 5. In another embodiment, the fusion polypeptide comprises one or more CDR(s) of G1. In still other embodiments, the fusion polypeptide comprises CDR H3 and/or CDR L3 of antibody G1. For purposes of this invention, an G1 fusion protein contains one or

more G1 antibodies and another amino acid sequence to which it is not attached in the native molecule, for example, a heterologous sequence or a homologous sequence from another region. Exemplary heterologous sequences include, but are not limited to a "tag" such as a FLAG tag or a 6His tag. Tags are well known in the art.

[0176] A G1 fusion polypeptide can be created by methods known in the art, for example, synthetically or recombinantly. Typically, the G1 fusion proteins are made by preparing an expressing a polynucleotide encoding them using recombinant methods described herein, although they may also be prepared by other means known in the art, including, for example, chemical synthesis.

Examples

Example 1: Generation and characterization of monoclonal antibodies directed against CGRP

[0177] Generation of anti-CGRP antibodies. To generate anti-CGRP antibodies that have cross-species reactivity for rat and human CGRP, mice were immunized with 25-100 µg of human α-CGRP or β-CGRP conjugated to KLH in adjuvant (50 µl per footpad, 100 µl total per mouse) at various intervals. Immunization was generally performed as described in Geerligs HJ et al., 1989, J. Immunol. Methods 124:95-102; Kenney JS et al., 1989, J. Immunol. Methods 121:157-166; and Wicher K et al., 1989, Int. Arch. Allergy Appl. Immunol. 89:128-135 . Mice were first immunized with 50 µg of human α-CGRP or β-CGRP conjugated to KLH in CFA (complete Freund's adjuvant). After 21 days, mice were secondly immunized with 25 µg of human β-CGRP (for mice first immunized with human α-CGRP) or α-CGRP (for mice first immunized with human β-CGRP) conjugated to KLH in IFA (incomplete Freund's adjuvant). Twenty three days later after the second immunization, third immunization was performed with 25 µg of rat α-CGRP conjugated to KLH in IFA. Ten days later, antibody titers were tested using ELISA. Forth immunization was performed with 25 µg of the peptide (rat α-CGRP-KLH) in IFA 34 days after the third immunization. Final booster was performed with 100 µg soluble peptide (rat α-CGRP) 32 days after the forth immunization.

[0178] Splenocytes were obtained from the immunized mouse and fused with NSO myeloma cells at a ratio of 10:1, with polyethylene glycol 1500. The hybrids were plated out into 96-well plates in DMEM containing 20% horse serum and 2-oxaloacetate/pyruvate/insulin (Sigma), and hypoxanthine/aminopterin/thymidine selection was begun. On day 8, 100 µl of DMEM containing 20% horse serum was added to all the wells. Supernatants of the hybrids were screened by using antibody capture immunoassay. Determination of antibody class was done with class-specific second antibodies.

[0179] A panel of monoclonal antibody-producing cell lines was selected based on their

binding to human and rat CGRP for further characterization. These antibodies and characteristics are shown below in Tables 2 and 3.

[0180] Purification and Fab fragment preparation. Monoclonal antibodies selected for further characterization were purified from supernatants of hybridoma cultures using protein A affinity chromatography. The supernatants were equilibrated to pH 8. The supernatants were then loaded to the protein A column MabSelect (Amersham Biosciences # 17-5199-02) equilibrated with PBS to pH 8. The column was washed with 5 column volumes of PBS, pH 8. The antibodies were eluted with 50 mM citrate-phosphate buffer, pH 3. The eluted antibodies were neutralized with 1M Phosphate Buffer, pH 8. The purified antibodies were dialyzed with PBS, pH 7.4. The antibody concentrations were determined by SDS-PAGE, using a murine monoclonal antibody standard curve.

[0181] Fabs were prepared by papain proteolysis of the full antibodies using Immunopure Fab kit (Pierce # 44885) and purified by flow through protein A chromatography following manufacturer instructions. Concentrations were determined by ELISA and/or SDS-PAGE electrophoresis using a standard Fab of known concentration (determined by amino acid analysis), and by A280 using 10D=0.6 mg/ml (or theoretical equivalent based on the amino acid sequence).

[0182] Affinity determination of the Fabs. Affinities of the anti-CGRP monoclonal antibodies were determined at either 25°C or 37°C using the Biacore3000™ surface plasmon resonance (SPR) system (Biacore, INC, Piscataway NJ) with the manufacture's own running buffer, HBS-EP (10 mM HEPES pH 7.4, 150 mM Nacl, 3 mM EDTA, 0.005% v/v polysorbate P20). Affinity was determined by capturing N-terminally biotinylated CGRP peptides (custom ordered from GenScript Corporation, New Jersey or Global Peptide Services, Colorado) via pre-immobilized streptavidin on SA chip and measuring binding kinetics of antibody Fab titrated across the CGRP surface. Biotinylated CGRP was diluted into HBS-EP and injected over the chip at a concentration of less than 0.001 mg/ml. Using variable flow time across the individual chip channels, two ranges of antigen density were achieved: <50 response units (RU) for detailed kinetic studies and about 800 RU for concentration studies and screening. Two- or three-fold serial dilutions typically at concentrations spanning 1 μM - 0.1 nM (aimed at 0.1-10x estimated K_D) of purified Fab fragments were injected for 1 minute at 100 μL/min and dissociation times of 10 minutes were allowed. After each binding cycle, surfaces were regenerated with 25 mM NaOH in 25% v/v ethanol, which was tolerated over hundreds of cycles. Kinetic association rate (k_{on}) and dissociation rate (k_{off}) were obtained simultaneously by fitting the data to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B. (1994). Methods Enzymology 6. 99-110) using the BIAevaluation program. Global equilibrium dissociation constants (K_D) or "affinities" were calculated from the ratio $K_D = k_{off}/k_{on}$. Affinities of the murine Fab fragments are shown in Tables 2 and 3.

[0183] Epitope mapping of the murine anti-CGRP antibodies. To determine the epitope that anti-CGRP antibodies bind on human α-CGRP, binding affinities of the Fab fragments to various CGRP fragments were measured as described above by capturing N-terminally

biotinylated CGRP fragments amino acids 19-37 and amino acids 25-37 on a SA sensor chip. Figure 1 shows their binding affinities measured at 25°C. As shown in Figure 1, all antibodies, except antibody 4901, bind to human α-CGRP fragments 19-37 and 25-37 with affinity similar to their binding affinity to full length human α-CGRP (1-37). Antibody 4901 binds to human α-CGRP fragment 25-37 with six fold lower affinity than binding to full length human α-CGRP fragment, due mainly to a loss in off-rate. The data indicate that these anti-CGRP antibodies generally bind to the C-terminal end of CGRP.

[0184] Alanine scanning was performed to further characterize amino acids in human α-CGRP involved in binding of anti-CGRP antibodies. Different variants of human α-CGRP with single alanine substitutions were generated by peptide synthesis. Their amino acid sequences are shown in Table 4 along with all the other peptides used in the Biacore analysis. Affinities of Fab fragments of the anti-CGRP antibodies to these variants were determined using Biacore as described above. As shown in Figure 1, all 12 antibodies target a C-terminal epitope, with amino acid F37 being the most crucial residue. Mutation of F37 to alanine significantly lowered the affinity or even completely knocked out binding of the anti-CGRP antibodies to the peptide. The next most important amino acid residue is G33, however, only the high affinity antibodies (7E9, 8B6, 10A8, and 7D11) were affected by alanine replacement at this position. Amino acid residue S34 also plays a significant, but lesser, role in the binding of these four high affinity antibodies.

Table 2. Characteristics of the anti-CGRP monoclonal antibodies' binding to human α-CGRP and their antagonist activity

Antibodies	K_D to human α-CGRP at 25°C (nM)	K_D to human α-CGRP at 37°C (nM)	Cell-based blocking human α-CGRP binding to its receptor at 25°C (measured by cAMP activation)	IC_{50} (nM binding sites) at 25°C (room temp.) measured in radioligand binding assay.
7E9	1.0	0.9	Yes	2.5
8B6	1.1	1.2	Yes	4.0
10A8	2.1	3.0	Yes	n.d.
7D11	4.4	5.4	Yes	n.d.
6H2	9.3	42	Yes	12.9
4901	61	139	Yes	58
14E10	80	179	Yes	n.d.
9B8	85	183	No	n.d.
13C2	94	379	No	n.d.
14A9	148	581	No	n.d.
6D5	210	647	No	n.d.
1C5	296	652	No	n.d.
Note: Antibody 4901 is commercially available (Sigma, Product No. C7113). n.d. = not determined				

Table 3. Characteristics of the anti-CGRP monoclonal antibodies' binding to rat α-CGRP and antagonist activity

Antibodies	K_D to rat α-CGRP at 37°C (nM)	Cell-based blocking of binding of rat α-CGRP to its receptor at 25°C (measured by cAMP activation)	In vivo blocking in saphenous nerve assay
4901	3.4	Yes	Yes
7E9	47	Yes	Yes
6H2	54	No	No
8B6	75	Yes	Yes
7D11	218	Yes	Yes
10A8	451	No	n.d.
9B8	876	No	n.d.
14E10	922	No	n.d.
13C2	> 1000	No	n.d.
14A9	> 1000	No	n.d.
6D5	> 1000	No	n.d.
1C5	> 1000	No	n.d.
"n.d." indicates no test was performed for the antibody.			

Table 4. Amino acid sequences of human α-CGRP fragments (SEQ ID NOS:15-40) and related peptides (SEQ ID NOS:41-47). All peptides are C-terminally amidated except SEQ ID NOS:36-40. Residues in bold indicate point mutations.

CGRP	Amino acid sequence	SEQ ID NO
1-37 (WT)	ACDTATCVTHRLAGLLSRSGGWKNNFVPTNVGSKAF	15
8-37	VTHRLAGLLSRSGGVVKNNFVPTNVGSKAF	16
19-37	SGGVVKNNFVPTNVGSKAF	17
P29A (19-37)	SGGVVKNNFVATNVGSKAF	18
K35A (19-37)	SGGVVKNNFVPTNVGSAAF	19
K35E (19-37)	SGGVVKNNFVPTNVGSEAF	20
K35M (19-37)	SGGVVKNNFVPTNVGSMAF	21
K35Q (19-37)	SGGVVKNNFVPTNVGSQAF	22
F37A (19-37)	SGGVVKNNFVPTNVGSKAA	23
25-38A	NNFVPTNVGSKAFA	24
25-37	NNFVPTNVGSKAF	25
F27A (25-37)	NNAVPTNVGSKAF	26
V28A (25-37)	NNFAPTNVGSKAF	27
P29A (25-37)	NNFVATNVGSKAF	28

CGRP	Amino acid sequence	SEQ ID NO
T30A (25-37)	NNFVPANVGSKAF	29
N31A (25-37)	NNFVPTAVGSKAF	30
V32A (25-37)	NNFVPTNAGSKAF	31
G33A (25-37)	NNFVPTNVASKAF	32
S34A (25-37)	NNFVPTNVGAKAF	33
F37A (25-37)	NNFVPTNVGSKAA	34
26-37	NFVPTNVGSKAF	35
19-37-COOH	SGGVVKNNFVPTNVGSKAF	36
19-36-COOH	SGGVVKNNFVPTNVGSKA	37
1-36-COOH	ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKA	38
1-19-COOH	ACDTATCVTHRLAGLLSRS	39
1-13-COOH	ACDTATCVTHRLA	40
rat α (1-37)	SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSEAF	41
rat α (19-37)	SGGVVKDNFVPTNVGSEAF	42
human β (1-37)	ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF	43
rat β (1-37)	SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSKAF	44
Human calcitonin (1-32)	CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP	45
Human amylin (1-37)	KCNTATCATQRLANFLVHSSNNFGAILSSTNVGSNTY	46
Human adrenomedullin (1-52)	YRQSMNNFQGLRSFGCRFGTCTVQKLAHQIYQFTDK DKDNVAPRSKISPQGY	47

Example 2: Screening of anti-CGRP antagonist antibodies using in vitro assays.

[0185] Murine anti-CGRP antibodies were further screened for antagonist activity in vitro using cell based cAMP activation assay and binding assay.

[0186] Antagonist activity measured by cAMP assay. Five microliters of human or rat α-CGRP (final concentration 50 nM) in the presence or absence of an anti-CGRP antibody (final concentration 1-3000 nM), or rat α-CGRP or human α-CGRP (final concentration 0.1 nM-10 μM; as a positive control for c-AMP activation) was dispensed into a 384-well plate (Nunc, Cat. No. 264657). Ten microliters of cells (human SK-N-MC if human α-CGRP is used, or rat L6 from ATCC if rat α-CGRP is used) in stimulation buffer (20 mM HEPES, pH 7.4, 146 mM Nacl,

5 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 500 uM 3-Isobutyl-1-methylxanthine (IBMX)) were added into the wells of the plate. The plate was incubated at room temperature for 30 min.

[0187] After the incubation, cAMP activation was performed using HitHunter™ Enzyme Fragment Complementation Assay (Applied Biosystems) following manufacture's instruction. The assay is based on a genetically engineered β-galactosidase enzyme that consists of two fragments -termed Enzyme Acceptor (EA) and Enzyme Donor (ED). When the two fragments are separated, the enzyme is inactive. When the fragments are together they can recombine spontaneously to form active enzyme by a process called complementation. The EFC assay platform utilizes an ED-cAMP peptide conjugate in which cAMP is recognized by anti-cAMP. This ED fragment is capable of reassociation with EA to form active enzyme. In the assay, anti-cAMP antibody is optimally titrated to bind ED-cAMP conjugate and inhibit enzyme formation. Levels of cAMP in cell lysate samples compete with ED-cAMP conjugate for binding to the anti-cAMP antibody. The amount of free ED conjugate in the assay is proportional to the concentration of cAMP. Therefore, cAMP is measured by the formation of active enzyme that is quantified by the turnover of β-galactosidase luminescent substrate. The cAMP activation assay was performed by adding 10 µl of lysis buffer and anti-cAMP antibody (1:1 ratio) following by incubation at room temperature for 60 min. Then 10 µl of ED-cAMP reagent was added into each well and incubated for 60 minutes at room temperature. After the incubation, 20 µl of EA reagent and CL mixture (containing the substrate) (1:1 ratio) was added into each well and incubated for 1-3 hours or overnight at room temperature. The plate was read at 1 second/well on PMT instrument or 30 seconds/place on imager. The antibodies that inhibit activation of cAMP by α-CGRP were identified (referred to as "yes") in Tables 2 and 3 above. Data in Tables 2 and 3 indicate that antibodies that demonstrated antagonist activity in the assay generally have high affinity. For example, antibodies having K_D (determined at 25°C) of about 80 nM or less to human α-CGRP or having K_D (determined at 37°C) of about 47 nM or less to rat α-CGRP showed antagonist activity in this assay.

[0188] Radioligand binding assay. Binding assay was performed to measure the IC_{50} of anti-CGRP antibody in blocking the CGRP from binding to the receptor as described previously. Zimmermann et al., Peptides 16:421-4, 1995; Mallee et al., J. Biol. Chem. 277:14294-8, 2002. Membranes (25 µg) from SK-N-MC cells were incubated for 90 min at room temperature in incubation buffer (50 mM Tris-HCL, pH 7.4, 5 mM $MgCL_2$, 0.1% BSA) containing 10 pM ^{125}I-human α-CGRP in a total volume of 1 mL. To determine inhibition concentrations (IC_{50}), antibodies or unlabeled CGRP (as a control), from a about 100 fold higher stock solution were dissolved at varying concentrations in the incubation buffer and incubated at the same time with membranes and 10 pM ^{125}I-human α-CGRP. Incubation was terminated by filtration through a glass microfiber filter (GF/B, 1µm) which had been blocked with 0.5% polyethylemimine. Dose response curves were plotted and K_i values were determined by using the equation: $K_i = IC_{50}/(1+([ligand]/K_D)$; where the equilibrium dissociation constant K_D = 8 pM for human α-CGRP to CGRP1 receptor as present in SK-N-MC cells, and B_{max} = 0.025 pmol/mg protein. The reported IC_{50} value (in terms of IgG molecules) was converted to binding

sites (by multiplying it by 2) so that it could be compared with the affinities (K_D) determined by Biacore (see Table 2).

[0189] Table 2 shows the IC_{50} of murine antibodies 7E9, 8B6, 6H2 and 4901. Data indicate that antibody affinity generally correlates with IC_{50}: antibodies with higher affinity (lower K_D values) have lower IC_{50} in the radioligand binding assay.

Example 3: Effect of anti-CGRP antagonist antibodies on skin vasodilatation induced by stimulation of rat saphenous nerve

[0190] To test antagonist activity of anti-CGRP antibodies, effect of the antibodies on skin vasodilatation by stimulation of rat saphenous nerve was tested using a rat model described previously. Escott et al., Br. J. Pharmacol. 110:772-776, 1993. In this rat model, electrical stimulation of saphenous nerve induces release of CGRP from nerve endings, resulting in an increase in skin blood flow. Blood flow in the foot skin of male Sprague Dwaley rats (170-300 g, from Charles River Hollister) was measured after saphenous nerve stimulation. Rats were maintained under anesthesia with 2% isoflurane. Bretylium tosylate (30 mg/kg, administered i.v.) was given at the beginning of the experiment to minimize vasoconstriction due to the concomitant stimulation of sympathetic fibers of the saphenous nerve. Body temperature was maintained at 37°C by the use of a rectal probe thermostatically connected to a temperature controlled heating pad. Compounds including antibodies, positive control (CGRP 8-37), and vehicle (PBS, 0.01% Tween 20) were given intravenously through the right femoral vein, except for the experiment shown in Figure 3, the test compound and the control were injected through tail vein, and for experiments shown in Figures 2A and 2B, antibodies 4901 and 7D11 were injected intraperitoneally (IP). Positive control compound CGRP 8-37 (vasodilatation antagonist), due to its short half-life, was given 3-5 min before nerve stimulation at 400 nmol/kg (200 µl). Tan et al., Clin. Sci. 89:656-73, 1995. The antibodies were given in different doses (1 mg/kg, 2.5 mg/kg, 5 mg/kg, 10 mg/kg, and 25 mg/kg).

[0191] For experiments shown in Figures 2A and 2B, antibody 4901 (25 mg/kg), antibody 7D11 (25 mg/kg), or vehicle control (PBS with 0.01% Tween 20) was administered intraperitoneally (IP) 72 hours before the electrical pulse stimulation. For experiment shown in Figure 3, antibody 4901 (1 mg/kg, 2.5 mg/kg, 5 mg/kg, or 25 mg/kg) or vehicle control (PBS with 0.01% Tween 20) was administered intravenously 24 hours before the electrical pulse stimulation. After administration of the antibodies or vehicle control, the saphenous nerve of the right hindlimb was exposed surgically, cut proximally and covered with plastic wrap to prevent drying. A laser Doppler probe was placed over the medio-dorsal side of the hindpaw skin, which is the region innervated by the saphenous nerve. Skin blood flow, measured as blood cell flux, was monitored with a laser Doppler flow meter. When a stable base-line flux (less than 5% variation) was established for at least 5 min, the nerve was placed over platinum bipolar electrodes and electrically stimulated with 60 pulses (2 Hz, 10 V, 1 ms, for 30 sec) and then again 20 minutes later. Cumulative change in skin blood flow was estimated by the area

under the flux-time curve (AUC, which is equal to change in flux multiplied by change in time) for each flux response to electrical pulse stimulation. The average of the blood flow response to the two stimulations was taken. Animals were kept under anesthesia for a period of one to three hours.

[0192] As shown in Figure 2A and Figure 2B, blood flow increase stimulated by applying electronic pulses on saphenous nerve was inhibited by the presence of CGRP 8-37 (400 nmol/kg, administered i.v.), antibody 4901 (25 mg/kg, administered ip), or antibody 7D11 (25 mg/kg, administered ip) as compared to the control. CGRP 8-37 was administered 3-5 min before the saphenous nerve stimulation; and antibodies were administered 72 hours before the saphenous nerve stimulation. As shown in Figure 3, blood flow increase stimulated by applying electronic pulses on saphenous nerve was inhibited by the presence of antibody 4901 at different doses (1 mg/kg, 2.5 mg/kg, 5 mg/kg, and 25 mg/kg) administered intravenously at 24 h before the saphenous nerve stimulation.

[0193] For experiments shown in Figures 4A and 4B, saphenous nerve was exposed surgically before antibody administration. The saphenous nerve of the right hindlimb was exposed surgically, cut proximally and covered with plastic wrap to prevent drying. A laser Doppler probe was placed over the medio-dorsal side of the hindpaw skin, which is the region innervated by the saphenous nerve. Skin blood flow, measured as blood cell flux, was monitored with a laser Doppler flow meter. Thirty to forty five minutes after bretylium tosylate injection, when a stable base-line flux (less than 5% variation) was established for at least 5 min, the nerve was placed over platium bipolar electrodes and electrically stimulated (2Hz, 10V, 1 ms, for 30 sec) and again 20 minutes later. The average of the blood flow flux response to these two stimulations was used to establish the baseline response (time 0) to electrical stimulation. Antibody 4901 (1 mg/kg or 10 mg/kg), antibody 7E9 (10 mg/kg), antibody 8B6 (10 mg/kg), or vehicle (PBS with 0.01% Tween 20) were then administered intravenously (i.v.). The nerve was subsequently stimulated (2Hz, 10V, 1 ms, for 30 sec) at 30 min, 60 min, 90 min, and 120 min after antibody or vehicle administration. Animals were kept under anesthesia for a period of approximately three hours. Cumulative change in skin blood flow was estimated by the area under the flux-time curve (AUC, which is equal to change in flux multiplied by change in time) for each flux response to electrical pulse stimulations.

[0194] As shown in Figure 4A, blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody 4901 1 mg/kg administered i.v., when electronic pulse stimulation was applied at 60 min, 90 min, and 120 min after the antibody administration, and blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody 4901 10 mg/kg administered i.v., when electronic pulse stimulation was applied at 30 min, 60 min, 90 min, and 120 min after the antibody administration. Figure 4B shows that blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody 7E9 (10 mg/kg, administered i.v.) when electronic pulse stimulation was applied at 30 min, 60 min, 90 min, and 120 min after antibody administration, and by the presence of antibody 8B6 (10 mg/kg, administered i.v.) when electronic pulse stimulation was

applied at 30 min after antibody administration.

[0195] These data indicate that antibodies 4901, 7E9, 7D11, and 8B6 are effective in blocking CGRP activity as measured by skin vasodilatation induced by stimulation of rat saphenous nerve.

Example 4. Characterization of anti-CGRP antibody G1 and its variants

[0196] Amino acid sequences for the heavy chain variable region and light chain variable region of anti-CGRP antibody G1 are shown in Figure 5. The following methods were used for expression and characterization of antibody G1 and its variants.

[0197] Expression vector used. Expression of the Fab fragment of the antibodies was under control of an IPTG inducible lacZ promoter similar to that described in Barbas (2001) Phage display: a laboratory manual, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press pg 2.10. Vector pComb3X), however, modifications included addition and expression of the following additional domains: the human Kappa light chain constant domain and the CH1 constant domain of IgG2 human immunoglobulin, Ig gamma-2 chain C region, protein accession number P01859; Immunoglobulin kappa light chain (homosapiens), protein accession number CAA09181.

[0198] Small scale Fab preparation. From E. Coli transformed (either using electroporation-competent TG1 cells or chemically-competent Top 10 cells) with a Fab library, single colonies were used to inoculate both a master plate (agar LB + carbenicillin (50 ug/mL) + 2% glucose) and a working plate (2 mL/well, 96-well/plate) where each well contained 1.5mL LB + carbenicillin (50 ug/mL) + 2% glucose. A gas permeable adhesive seal (ABgene, Surrey, UK) was applied to the plate. Both plates were incubated at 30°C for 12-16h; the working plate was shaken vigorously. The master plate was stored at 4°C until needed, while the cells from the working plate were pelleted (4000 rpm, 4°C, 20 mins) and resuspended in 1.0 mL LB + carbenicillin (50 ug/mL) + 0.5 mM IPTG to induce expression of Fabs by vigorous shaking for 5h at 30°C. Induced cells were centrifuges at 4000 rpm, 4°C for 20 mins and resuspended in 0.6 mL Biacore HB-SEP buffer (10 mM Hepes pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v P20). Lysis of HB-SEP resuspended cells was accomplished by freezing (-80°C) and then thawing at 37°C. Cell lysates were centrifuged at 4000 rpm, 4°C for 1 hour to separate the debris from the Fab-containing supernatants, which were subsequently filtered (0.2 um) using a Millipore MultiScreen Assay System 96-Well Filtration Plate and vacuum manifold. Biacore was used to analyze filtered supernatants by injecting them across CGRPs on the sensor chip. Affinity-selected clones expressing Fabs were rescued from the master plate, which provided template DNA for PCR, sequencing, and plasmid preparation.

[0199] Large scale Fab preparation. To obtain kinetic parameters, Fabs were expressed on a larger scale as follows. Erlenmeyer flasks containing 150 mL LB + carbenicillin (50 ug/mL) + 2% glucose were inoculated with 1 mL of a "starter" overnight culture from an affinity-selected

Fab-expressing E. Coli clone. The remainder of the starter culture (~3 mL) was used to prepare plasmid DNA (QIAprep mini-prep, Qiagen kit) for sequencing and further manipulation. The large culture was incubated at 30°C with vigorous shaking until an OD_{600nm} of 1.0 was attained (typically 12-16 h). The cells were pelleted by centrifuging at 4000 rpm, 4°C for 20mins, and resuspended in 150 mL LB + carbenicillin (50 ug/mL) + 0.5 mM IPTG. After 5 h expression at 30°C, cells were pelleted by centrifuging at 4000 rpm, 4°C for 20mins, resuspended in 10 mL Biacore HBS-EP buffer, and lysed using a single freeze (-80°C)/thaw (37°C) cycle. Cell lysates were pelleted by centrifuging at 4000rpm, 4°C for 1hour, and the supernatant was collected and filtered (0.2um). Filtered supernatants were loaded onto Ni-NTA superflow sepharose (Qiagen, Valencia. CA) columns equilibrated with PBS, pH 8, then washed with 5 column volumes of PBS, pH 8. Individual Fabs eluted in different fractions with PBS (pH 8) + 300 mM Imidazole. Fractions containing Fabs were pooled and dialyzed in PBS, then quantified by ELISA prior to affinity characterization.

[0200] Full antibody preparation. For expression of full antibodies, heavy and light chain variable regions were cloned in mammalian expression vectors and transfected using lipofectamine into HEK 293 cells for transient expression. Antibodies were purified using protein A using standard methods.

[0201] Vector pDb.CGRP.hFcGI is an expression vector comprising the heavy chain of the G1 antibody, and is suitable for transient or stable expression of the heavy chain. Vector pDb.CGRP.hFcGI has nucleotide sequences corresponding to the following regions: the murine cytomegalovirus promoter region (nucleotides 7-612); a synthetic intron (nucleotides 613-1679); the DHFR coding region (nucleotides 688-1253); human growth hormone signal peptide (nucleotides 1899-1976); heavy chain variable region of G1 (nucleotides 1977-2621); human heavy chain IgG2 constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence; see Eur. J. Immunol. (1999) 29:2613-2624). Vector pDb.CGRP.hFcGI was deposited at the ATCC on July 15, 2005, and was assigned ATCC Accession No. PTA-6867.

[0202] Vector pEb.CGRP.hKGI is an expression vector comprising the light chain of the G1 antibody, and is suitable for transient expression of the light chain. Vector pEb.CGRP.hKGI has nucleotide sequences corresponding to the following regions: the murine cytomegalovirus promoter region (nucleotides 2-613); human EF-1 intron (nucleotides 614-1149); human growth hormone signal peptide (nucleotides 1160-1237); antibody G1 light chain variable region (nucleotides 1238-1558); human kappa chain constant region (nucleotides 1559-1882). Vector pEb.CGRP.hKGI was deposited at the ATCC on July 15, 2005, and was assigned ATCC Accession No. PTA-6866.

[0203] Biacore assay for affinity determination. Affinities of G1 monoclonal antibody and its variants were determined at either 25°C or 37°C using the Biacore3000™ surface plasmon resonance (SPR) system (Biacore, INC, Piscataway NJ). Affinity was determined by capturing N-terminally biotinylated CGRP or fragments via pre-immobilized streptavidin (SA sensor chip) and measuring the binding kinetics of antibody G1 Fab fragments or variants titrated across

the CGRP or fragment on the chip. All Biacore assays were conducted in HBS-EP running buffer (10 mM HEPES pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% v/v polysorbate P20). CGRP surfaces were prepared by diluting the N-biotinylated CGRP to a concentration of less than 0.001 mg/mL into HBS-EP buffer and injecting it across the SA sensor chip using variable contact times. Low capacity surfaces, corresponding to capture levels <50 response units (RU) were used for high-resolution kinetic studies, whereas high capacity surfaces (about 800 RU of captured CGRP) were used for concentration studies, screening, and solution affinity determinations. Kinetic data were obtained by diluting antibody G1 Fab serially in two- or three-fold increments to concentrations spanning 1uM-0.1nM (aimed at 0.1-10x estimated K_D). Samples were typically injected for 1 minute at 100 µL/min and dissociation times of at least 10 minutes were allowed. After each binding cycle, surfaces were regenerated with 25mM NaOH in 25% v/v ethanol, which was tolerated over hundreds of cycles. An entire titration series (typically generated in duplicate) was fit globally to a 1:1 Langmuir binding model using the BIAevaluation program. This returned a unique pair of association and dissociation kinetic rate constants (respectively, k_{on} and k_{off}) for each binding interaction, whose ratio gave the equilibrium dissociation constant ($K_D = k_{off}/k_{on}$). Affinities (K_D values) determined in this way are listed in Tables 6 and 7.

[0204] High-resolution analysis of binding interactions with extremely slow offrates. For interactions with extremely slow offrates (in particular, antibody G1 Fab binding to human □-CGRP on the chip at 25°C), affinities were obtained in a two-part experiment. The protocol described above was used with the following modifications. The association rate constant (k_{on}) was determined by injecting a 2-fold titration series (in duplicate) spanning 550 nM-1 nM for 30 sec at 100 uL/min and allowing only a 30 sec dissociation phase. The dissociation rate constant (k_{off}) was determined by injecting three concentrations (high, medium, and low) of the same titration series in duplicate for 30sec and allowing a 2-hour dissociation phase. The affinity (K_D) of each interaction was obtained by combining the k_{on} and k_{off} values obtained in both types of experiments, as shown in Table 5.

[0205] Determining solution affinity by Biacore. The solution affinity of antibody G1 for rat α-CGRP and F37A (19-37) human α-CGRP was measured by Biacore at 37°C. A high capacity CGRP chip surface was used (the high-affinity human α-CGRP was chosen for detection purposes) and HBS-EP running buffer was flowed at 5 uL/min. Antibody G1 Fab fragment at a constant concentration of 5 nM (aimed to be at or below the expected K_D of the solution-based interaction) was pre-incubated with competing peptide, either rata-CGRP or F37A (19-37) human α-CGRP, at final concentrations spanning 1 nM to 1 uM in 3-fold serial dilutions. Antibody G1 Fab solutions in the absence or presence of solution-based competing peptide, were injected across CGRP on the chip and the depletion of binding responses detected at the chip surface as a result of solution competition was monitored. These binding responses were converted to "free Fab concentrations" using a calibration curve, which was constructed by titrating antibody G1 Fab alone (5, 2.5, 1.25, 0.625, 0.325 and 0 nM) across the CGRP on the chip. "Free Fab concentrations" were plotted against the concentration of competing solution-based peptide used to generate each data point and fit to a solution affinity model using the

BIAevaluation software. The solution affinities determined (indirectly) in this way are shown in Tables 5 and 7 and were used to validate the affinities obtained when Fabs are injected directly across N-biotinylated CGRPs on a SA chip. The close agreement between the affinities determined by these two methods confirms that tethering an N-biotinylated version of the CGRP to the chip does not alter its native solution binding activity.

[0206] Table 5 below shows the binding affinities of antibody G1 to human α-CGRP, human β-CGRP, rat α-CGRP, and rat β-CGRP determined by Biacore, by flowing Fab fragments across N-biotinylated CGRPs on a SA chip. To better resolve the affinities of binding interactions with extremely slow offrates, affinities were also determined in a two-part experiment to complement this assay orientation, the solution affinity of the rat α-CGRP interaction was also determined (as described above). The close agreement of the affinities measured in both assay orientations confirms that the binding affinity of the native rat α-CGRP in solution is not altered when it is N-biotinylated and tethered to a SA chip.

Table 5. Binding affinities of antibody G1 Fabs titrated across CGRPs on the chip

CGRP on chip	Temp. (°C)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
Human α-CGRP	25	1.86×10^5	7.80×10^{-6}	0.042 (7%, n=4)*
Human α-CGRP	37	5.78×10^5	3.63×10^{-5}	0.063 (4%, n=2)*
Human β-CGRP	37	4.51×10^5	6.98×10^{-5}	0.155
Rat α-CGRP	25	5.08×10^4	6.18×10^{-5}	1.22 (12%, n=2)*
Rat α-CGRP	37	1.55×10^5	3.99×10^{-4}	2.57* (Solution K_D=10 (50%, n=4)**
Rat β-CGRP	37	5.16×10^5	7.85×10^{-5}	0.152

*Affinities for α-CGRPs (rat and human) were determined in a high-resolution two-part experiment, in which the dissociation phase was monitored for 2 hours (the values for k_{on}, k_{off}, and K_D represent the average of n replicate experiments with the standard deviation expressed as a percent variance). Affinities for β-CGRPs (rat and human) were determined by global analysis using only a 20-min dissociation phase, which was not accurate enough to quantify their extremely offrates (their offrates are likely slower than stated here and therefore their affinities are likely even higher). Antibody G1 Fab dissociated extremely slowly from all CGRPs (except α-rat CGRP) with offrates that approached the resolution limit of the Biacore assay (especially at 25°C).
**Solution affinity determined by measuring the depletion of binding responses detected at CGRP on the chip for antibody G1 Fab pre-incubated with solution-based rat α-CGRP competitor.

[0207] Table 6 below shows antibodies having the amino acid sequence variation as compared to antibody G1 and their affinities to both rat α-CGRP and human α-CGRP. All amino

acid substitutions of the variants shown in Table 6 are described relative to the sequence of G1. The binding affinities of Fab fragments were determined by Biacore by flowing them across CGRPs on a SA chip.

Table 6. Amino acid sequences and binding affinity data for antibody G1 variants determined at 37°C by Biacore.

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
G1					$3.99x10^{-4}$	2.57	$3.63 x10^{-5}$	0.063
M1				A100L	$1.10x10^{-3}$		$1.73x10^{-4}$	
M2				L99A	$2.6x10^{-3}$	58	$3.1x10^{-4}$	3
				A100R				
M3				L99A	$2.0x10^{-3}$	61	$2.1x10^{-4}$	1.7
				A100S				
M4				L99A	$1.52x10^{-3}$	84.4	$6.95x10^{-5}$	0.43
				A100V				
M5				L99A	$7.35x10^{-4}$	40.8	$3.22x10^{-5}$	0.20
				A100Y				
M6				L99N	$7.84.x10^{-4}$	43.6	$1.33x10^{-4}$	0.83
M7				L99N	$9.18x10^{-4}$	51.0	$2.43x10^{-4}$	1.52
				A100C				
M8				L99N	$7.45x10^{-4}$	41.4	$9.20x10^{-5}$	0.58
				A100G				
M9				L99N	n.d.	n.d.	$1.00x10^{-5}$	0.06
				A100Y				
M10				L99S	$1.51x10^{-3}$	83.9	$1.73x10^{-4}$	1.08
				A100S				
M11				L99S	$4.83x10^{-3}$	268.3	$2.83x10^{-4}$	1.77
				A100T				
M12				L99S	$1.94x10^{-3}$	107.8	$1.01x10^{-4}$	0.63
				A100V				
M13				L99T	$1.84x10^{-3}$	102.2	$1.86x10^{-4}$	1.16
				A100G				
M14				L99T	n.d.	n.d.	$1.00x10^{-5}$	0.06
				A100K				

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
M15				L99T	$1.15x10^{-3}$	63.9	$1.58x10^{-5}$	0.10
				A100P				
M16				L99T	$9.96x10^{-4}$	55.3	$1.65x10^{-4}$	1.03
				A100S				
M17				L99T	$2.06x10^{-3}$	114.4	$1.85x10^{-4}$	1.16
				A100V				
M18				L99V	$1.22x10^{-3}$	67.8	$7.03x10^{-5}$	0.44
				A100G				
M19				L99V	n.d.	n.d.	$1.00x10^{-5}$	0.06
				A100R				
M20	R28W			L99R	$1.44x10^{-3}$	80.0	$1.36x10^{-4}$	0.85
				A100L				
M21	R28W			L99S	$6.95x10^{-4}$	15.2	$1.42x10^{-4}$	1.23
M22	R28W			L99T	$1.10x10^{-3}$	61.1	$1.16x10^{-4}$	0.73
M23	R28G			L99T	$7.99x10^{-4}$	44.4	$1.30x10^{-4}$	0.81
				A100V				
M24	R28L			L99T	$1.04x10^{-3}$	57.8	$1.48x10^{-4}$	0.93
				A100V				
M25	R28N			L99T	$1.4x10^{-3}$	76	$1.4x10^{-4}$	1.3
				A100V				
M26	R28N		A57G	L99T	$9.24x10^{-4}$	51.3	$1.48x10^{-4}$	0.93
				A100V				
M27	R28N			L99T	$3.41x10^{-3}$	189.4	$3.57x10^{-4}$	2.23
	T30A			A100V				
M28	R28N		E54R	L99T	$1.25x10^{-3}$	69.4	$9.96x10^{-5}$	0.62
	T30D		A57N	A100V				
M29	R28N			L99T	$3.59x10^{-3}$	199.4	$3.80x10^{-4}$	2.38
	T30G			A100V				
M30	R28N		E54K	L99T	$6.38x10^{-3}$	354.4	$5.90x10^{-4}$	3.69
	T30G		A57E	A100V				

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
M31	R28N		E54K	L99T	$3.61x10^{-3}$	200.6	$3.47x10^{-4}$	2.17
	T30G		A57G	A100V				
M32	R28N		E54K	L99T	$2.96x10^{-3}$	164.4	$2.71x10^{-4}$	1.69
	T30G		A57H	A100V				
M33	R28N		E54K	L99T	$9.22x10^{-3}$	512.2	$7.50x10^{-4}$	4.69
	T30G		A57N	A100V				
			S58G					
M34	R28N		E54K	L99T	$2.17x10^{-3}$	120.6	$6.46x10^{-4}$	4.04
	T30G		A57N	A100V				
			S58T					
M35	R28N		E54K	L99T	$3.99x10^{-3}$	221.7	$3.39x10^{-4}$	2.12
	T30G		A57S	A100V				
M36	R28N			L99T	$4.79x10^{-3}$	266.1	$2.39x10^{-4}$	1.49
	T30R			A100V				
M37	R28N		A57G	L99T	$1.45x10^{-3}$	80.6	$2.26x10^{-4}$	1.41
	T30S			A100V				
M38	R28N			L99T	$5.11x10^{-3}$	283.9	$2.18x10^{-4}$	1.36
	T30W			A100V				
M39	R28N	G50A	A57N	L99T	$9.95x10^{-3}$	552.8	$4.25x10^{-4}$	2.66
		L56T	S58Y	A100V				
M40	R28N	G50A	E54K	L99T	0.36	20000.0	$1.28x10^{-3}$	8.00
		L56T	A57L	A100V				
M41	R28N	G50A	E54K	L99T	$4.53x10^{-3}$	251.7	$2.10x10^{-4}$	1.31
		L56T	A57N	A100V				
			E64D					
M42	R28N	G50A	E54K	L99T	$7.52x10^{-3}$	417.8	$4.17x10^{-4}$	2.61
		L56T	A57N	A100V				
			H61F					
M43	R28N	G50A	E54K	L99T	$4.53x10^{-3}$	251.7	$2.63x10^{-4}$	1.64
		L56T	A57N	A100V				
			S58C					
M44	R28N	G50A	E54K	L99T	$6.13x10^{-3}$	443	$2.10x10^{-4}$	2.05

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
		L56T	A57N	A100V				
			S58E					
M45	R28N	G50A	E54K	L99T	$5.58x10^{-3}$	259	$2.11x10^{-4}$	1.85
		L56T	A57N	A100V				
			S58E					
			E64D					
M46	R28N	G50A	E54K	L99T	$2.94x10^{-3}$	163.3	$5.39x10^{-4}$	3.37
		L56T	A57N	A100V				
			S58E					
			H61F					
M47	R28N	G50A	E54K	L99T	$8.23x10^{-3}$	457.2	$3.32x10^{-4}$	2.08
		L56T	A57N	A100V				
			S58G					
M48	R28N	G50A	E54K	L99T	0.0343	1905.6	$8.42x10^{-4}$	5.26
		L56T	A57N	A100V				
			S58L					
M49	R28N	G50A	E54K	L99T	0.0148	822.2	$5.95x10^{-4}$	3.72
		L56T	A57N	A100V				
			S58Y					
			H61F					
M50	R28N	G50A	E54K	L99T	$5.30x10^{-3}$	294.4	$4.06x10^{-4}$	2.54
		L56T	A57R	A100V				
M51	R28N	L56I	E54K	L99T	$1.18x10^{-3}$	65.6	$1.31x10^{-4}$	0.82
			A57G	A100V				
M52	R28N	L56I	E54K	L99T	$2.29x10^{-3}$	127.2	$2.81x10^{-4}$	1.76
			A57N	A100V				
			S58A					
M53	R28N	L561	E54K	L99T	$1.91x10^{-3}$	106.1	$3.74x10^{-4}$	2.34
			A57N	A100V				
			S58G					
M54	R28N	G50A	E54K	L99T	$2.16x10^{-3}$	120.0	$1.79x10^{-3}$	11.19
	T30A		A57N	A100V				

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
			S58P					
M55	R28N	L56S	E54K	L99T	$5.85x10^{-3}$	325.0	$4.78x10^{-4}$	2.99
	T30A		A57N	A100V				
			S58E					
			E64D					
M56	R28N	L56S	E54K	L99T	$9.35x10^{-3}$	519.4	$4.79x10^{-4}$	2.99
	T30D		A57N	A100V				
			H61F					
M57	R28N	L56S	E54K	L99T	0.0104	1,200	$3.22x10^{-4}$	3.08
	T30D		A57N	A100V				
			S58E					
M58	R28N	L56S	E54K	L99T	No binding	n.d.	$1.95x10^{-3}$	12.19
	T30D		A57N	A100V				
			S58I					
			H61F					
M59	R28N	L56S	E54K	L99T	0.0123	683.3	$5.24x10^{-4}$	3.28
	T30D		A57N	A100V				
			S58N					
			H61F					
M60	R28N	L56S	E54K	L99T	0.0272	1511.1	$9.11x10^{-4}$	5.69
	T30D		A57N	A100V				
			S58R					
			H61F					
M61	R28N	A51H	E54Q	L99T	$5.21x10^{-3}$	289.4	$4.59x10^{-4}$	2.87
	T30G		A57N	A100V				
			H61F					
M62	R28N	A51H	E54K	L99T	$5.75x10^{-3}$	242	$5.57x10^{-4}$	5.86
	T30G	L56T	A57N	A100V				
			S58E					
M63	R28N	G50A	E54K	L99T	$2.65x10^{-3}$	147.2	$1.50x10^{-3}$	9.38
	T30G		A57N	A100V				
			S58T					

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
M64	R28N	G50A	E54K	L99T	0.0234	1300.0	$1.32x10^{-3}$	8.25
	T30G		A57N	A100V				
			S58V					
M65	R28N	G50A	E54K	L99T	$4.07x10^{-3}$	226.1	$8.03X10^{-4}$	5.02
	T30G	L56I	A57C	A100V				
M66	R28N	L56I	E54K	L99T	$5.11x10^{-3}$	283.9	$5.20x10^{-4}$	3.25
	T30G		A57E	A100V				
M67	R28N	L56I	E54K	L99T	$1.71x10^{-3}$	95.0	$8.20x10^{-4}$	5.13
	T30G		A57F	A100V				
M68	R28N	L56I	E54K	L99T	$6.76x10^{-3}$	375.6	$4.28x10^{-4}$	2.68
	T30G		A57N	A100V				
			S58D					
			E64D					
M69	R28N	L56I	E54K	L99T	$1.81x10^{-3}$	100.6	$7.33x10^{-4}$	4.58
	T30G		A57N	A100V				
			S58E					
M70	R28N	L56I	E54K	L99T	$6.07x10^{-3}$	337.2	$5.59x10^{-4}$	3.49
	T30G		A57S	A100V				
M71	R28N	L56I	E54K	L99T	$2.12x10^{-3}$	117.8	$1.28x10^{-3}$	8.00
	T30G		A57Y	A100V				
M72	R28N	L56S	E54K	L99T	$3.95x10^{-3}$	219.4	$4.00x10^{-4}$	2.50
	T30G			A100V				
M73	R28N	L56S	E54K	L99T	$3.00x10^{-3}$	166.7	$2.55x10^{-4}$	1.59
	T30G		A57N	A100V				
			S58Y					
			E64D					
M74	R28N	L56S	E54K	L99T	$6.03x10^{-3}$	335.0	$5.97x10^{-4}$	3.73
	T30G		A57S	A100V				
M75	R28N	L56S	E54K	L99T	$1.87x10^{-2}$	1038.9	$1.16x10^{-3}$	7.25
	T30G		A57V	A100V				
M76	R28N	G50A	A57G	L99T	$1.16x10^{-3}$	64.4	$3.64x10^{-4}$	2.28
	T30S	L56T		A100V				

Clone	L1	L2	H2	HC-FW3	α-rat k_{off} (1/s)	α-rat K_D (nM)	α-human k_{off} (1/s)	α-human K_D (nM)
M77	R28N	G50A	E54K	L99T	0.0143	794.4	$4.77x10^{-4}$	2.98
	T30S	L56T	A57D	A100V				
M78	R28N	G50A	E54K	L99T	0.167	9277.8	$1.31x10^{-3}$	8.19
	T30S	L56T	A57N	A100V				
			S58T					
M79	R28N	G50A	E54K	L99T	0.19	10555.6	$1.29x10^{-3}$	8.06
	T30S	L56T	A57P	A100V				
M80	R28N	L56I	E54K	L99T	0.0993	5516.7	$2.09x10^{-3}$	13.06
	T30S		A57N	A100V				
			S58V					
M81	R28N	L56S	E54K	L99T	$4.29x10^{-3}$	238.3	$4.90x10^{-4}$	3.06
	T30S		A57N	A100V				
			S58E					
M82	R28N	A51H	A57N	L99T	$6.99x10^{-3}$	388.3	$8.77x10^{-4}$	5.48
	T30V	L56T		A100V				
M83	R28N	A51H	E54K	L99T	No binding	n.d.	$9.33x10^{-4}$	5.83
	T30V	L56T	A57N	A100V				
			S58M					
			H61F					
M84	R28N	A51H	E54N	L99T	$1.76x10^{-2}$	977.8	$1.08x10^{-3}$	6.75
	T30V	L56T	A57N	A100V				

All CDRs including both Kabat and Chothia CDRs. Amino acid residues are numbered sequentially (see Figure 5). All clones have L3+H1+H3 sequences identical to G1.
$K_D = k_{off}/k_{on}$. All k_{off} values were determined in a screening mode except those that are <u>underlined</u>, which were obtained by global analysis of a Fab concentration series (G1 was analyzed in a high-resolution mode). <u>Underlined</u> K_D values were therefore determined experimentally by measuring k_{on}. Other k_{on} values were estimated to be the same as M25.
n.d. = not determined

[0208] To determine the epitope on human α-CGRP that is recognized by antibody G1, Biacore assays described above were used. Human α-CGRP was purchased as an N-biotinylated version to enable its high-affinity capture via SA sensor chips. The binding of G1

Fab fragment to the human α-CGRP on the chip in the absence or presence of a CGRP peptide was determined. Typically, a 2000:1 mol peptide/Fab solution (e.g., 10 uM peptide in 50nM G1 Fab) was injected across human α-CGRP on the chip. Figure 6 shows the percentage of binding blocked by competing peptide. Data shown in Figure 6 indicate that peptides that block 100% binding of G1 Fab to human α-CGRP are 1-37 (WT), 8-37, 26-37, P29A (19-37), K35A (19-37), K35E (19-37), and K35M (19-37) of human α-CGRP; 1-37 of β-CGRP (WT); 1-37 of rat α-CGRP (WT); and 1-37 of rat β-CGRP (WT). All these peptides are amidated at the C-terminus. Peptides F37A (19-37) and 19-37 (the latter not amidated at the C-terminus) of human α-CGRP also blocked about 80% to 90% of binding of G1 Fab to human α-CGRP. Peptide 1-36 (not amidated at the C-terminus) of human α-CGRP blocked about 40% of binding of G1 Fab to human α-CGRP. Peptide fragment 19-36 (amidated at the C-terminus) of human α-CGRP; peptide fragments 1-13 and 1-19 of human α-CGRP (neither of which are amidated at the C-terminus); and human amylin, calcitonin, and adrenomedullin (all amidated at the C-terminus) did not compete with binding of G1 Fab to human α-CGRP on the chip. These data demonstrate that G1 targets a C-terminal epitope of CGRP and that both the identity of the most terminal residue (F37) and its amidation is important for binding.

[0209] Binding affinities of G1 Fab to variants of human α-CGRP (at 37°C) was also determined. Table 7 below shows the affinities as measured directly by titrating G1 Fab across N-biotinylated human α-CGRP and variants on the chip. Data in Table 7 indicate that antibody G1 binds to a C-terminal epitope with F37 and G33 being the most important residues. G1 does not bind to CGRP when an extra amino acid residue (alanine) is added at the C-terminal (which is amidated).

Table 7. Binding affinities of G1 Fab to human α-CGRP and variants measured at 37°C (see Table 4 for their amino acid sequences)

CGRP on chip	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
1-37 (WT)	4.68×10^{5}	7.63×10^{-5}	0.16 (high resolution K_D = 0.06)
19-37	4.60×10^{5}	7.30×10^{-5}	0.16
25-37	3.10×10^{5}	8.80×10^{-5}	0.28
F27A (25-37)	3.25×10^{5}	1.24×10^{-4}	0.38
V28A (25-37)	3.32×10^{5}	9.38×10^{-5}	0.28
P29A (25-37)	2.26×10^{5}	1.78×10^{-4}	0.79
T30A (25-37)	1.79×10^{5}	8.41×10^{-5}	0.47
N31A (25-37)	2.17×10^{5}	1.14×10^{-4}	0.53
V32A (25-37)	2.02×10^{5}	3.46×10^{-4}	1.71
G33A (25-37)	2.07×10^{5}	0.0291	141
S34A (25-37)	2.51×10^{5}	7.64×10^{-4}	3.04
K35A (19-37)	2.23×10^{5}	2.97×10^{-4}	1.33

CGRP on chip	k_{on} (1/Ms)	k_{off} (1/s)	K_D (nM)
K35E (19-37)	$5.95x10^4$	$5.79x10^{-4}$	9.73
K35M (19-37)	$2.63x10^5$	$1.34x10^{-4}$	0.51
K35Q (19-37)	$1.95x10^5$	$2.70x10^{-4}$	1.38
F37A (25-37)	$8.90x10^4$	$8.48x10^{-3}$	95 (solution K_D = 172 nM)
38A (25-38A)	-	-	No binding detected

[0210] The above data indicate that the epitope that antibody G1 binds is on the C-terminal end of human α-CGRP, and amino acids 33 and 37 on human α-CGRP are important for binding of antibody G1. Also, the amidation of residue F37 is important for binding.

Example 5: Effect of anti-CGRP antagonist antibody G1 on skin vasodilatation induced by stimulation of rat saphenous nerve

[0211] To test antagonist activity of anti-CGRP antibody G1, effect of the antibody on skin vasodilatation by stimulation of rat saphenous nerve was tested using a rat model described in Example 3. Briefly, rats were maintained anesthesia with 2% isoflurane. Bretylium tosylate (30 mg/kg, administered i.v.) was given at the beginning of the experiment to minimize vasoconstriction due to the concomitant stimulation of sympathetic fibers of the saphenous nerve. Body temperature was maintained at 37°C by the use of a rectal probe thermostatically connected to a temperature controlled heating blanket. The saphenous nerve of the right hindlimb was exposed surgically, cut proximally and covered with plastic wrap to prevent drying. A laser Doppler probe was placed over the medio-dorsal side of the hindpaw skin, which is the region innervated by the saphenous nerve. Skin blood flow, measured as blood cell flux, was monitored with a laser Doppler flow meter. In experiments to determine effects of antibody within two hours of injection thirty to forty five minutes after bretylium tosylate injection, when a stable base-line flux (less than 5% variation) was established for at least 5 min, the nerve was placed over platium bipolar electrodes and electrically stimulated (2Hz, 10V, 1 ms, for 30 sec) and again 20 minutes later. The average of the blood flow flux response to these two stimulations was used to establish the baseline response (time 0) to electrical stimulation. Antibody G1 (1 mg/kg or 10 mg/kg) or vehicle (PBS with 0.01% Tween 20 equal volume to 10 mg/kg G1) were then administered intravenously (i.v.). The nerve was subsequently stimulated (2Hz, 10V, 1 ms, for 30 sec) at 30 min, 60 min, 90 min, and 120 min after the antibody administration. Animals were kept under anesthesia for a period of approximately three hours. Cumulative change in skin blood flow was estimated by the area under the flux-time curve (AUC, which is equal to change in flux multiplied by change in time) for each flux response to electrical pulse stimulations.

[0212] As shown in Figure 7, blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody G1 at 1 mg/kg (administered i.v.) as compared to the vehicle, when the saphenous nerve was electrically stimulated at 90 min after the antibody administration. Blood flow increase stimulated by applying electronic pulses on saphenous nerve was significantly inhibited by the presence of antibody G1 at 10 mg/kg (administered i.v.) as compared to the vehicle, when the saphenous nerve was electrically stimulated at 90 minutes and 120 minutes after antibody administration.

[0213] In experiments to determine effects of the antibodies at longer time points in the saphenous assay, rats were injected i.v. with the indicated doses of antibody 24 hours or 7 days prior to preparing the animal for saphenous nerve stimulation as described above. In these experiments it was impossible to establish a baseline response in individual rats to electrical pulse stimulation prior to dosing, so treated groups were compared to animals dosed with vehicle (PBS, 0.01% Tween 20) at 24 hours or 7 days.

[0214] As shown in Figures 8A and 8B blood flow increases in the dorso-medial hindpaw skin evoked by saphenous nerve stimulation were significantly inhibited in the groups of animals dosed with either 10 mg/kg or 3 mg/kg G1 at either 24 hours or 7 days prior to stimulation as compared to vehicle groups dosed at the same time points.

[0215] Figure 8C represents a curve fit analysis applied to the dose response data represented in figures 8A and 8B to determine the dose required for 50% maximal effect (EC_{50}). The EC_{50} at 24 hours is 1.3 mg/kg and the EC_{50} at 7 days is slightly lower (0.8mg/kg).

Example 6: Acute effect of anti-CGRP antagonist antibody G1 in a dural artery (closed cranial window) assay

[0216] Closed Cranial Window Model: The purpose of this experiment was to determine the acute effect of anti-CGRP antagonist antibodies and compare it with the acute effect of the CGRP receptor antagonist BIBN4096BS. Experiments were carried out as previously described (Williamson et al., Cephalalgia 17(4):518-24 (1997)) with the following modifications. Sprague Dawley rats (300-400g) were anesthetized with 70mg/kg i.p. pentobarbital. Anesthesia was maintained with 20mg/kg/hr i.v. pentobarbital. Rats were cannulated through the jugular vein for delivery of all drugs. Blood pressure was monitored with a probe (mikro-tip catheter, Millar Instruments) threaded through the femoral artery into the abdominal aorta. The rats were tracheotomized and breathing rate was maintained at 75 breaths per minute at a volume of 3.5 mL. After fixating the head in a stereotactic instrument and removing the scalp, a 2x6mm window in the left parietal area just lateral to the sagittal suture was made by thinning the bone with a dental drill. Using a micromanipulator, a platinum bipolar electrode was lowered onto the surface and covered with heavy mineral oil. Lateral to the electrode window another window of 5x6 mm was created and filled with heavy mineral oil through which the diameter of a branch of the middle meningeal artery (MMA) was continuously monitored with a CCD camera and a video dimension analyzer (Living Systems). The rats were rested for no less than 45 minutes

after the preparation. A baseline response to electrical stimulation was established (15 V, 10 hz, 0.5 ms pulses, 30 seconds) and then rats were dosed i.v. with experimental compound (10mg/kg mu7E9, 300µg/kg BIBN4096BS or PBS 0.01%Tween 20). Additional electrical stimulations were done at 5 (BIBN4096BS), 30, 60, 90 and 120 minutes after dosing. All data was recorded using chart software (ADInstruments).

[0217] As shown in Figure 9 mu7E9 at 10mg/kg significantly blocks MMA dilation evoked by electrical field stimulation within 60 minutes after dosing and maintains the effect throughout the duration of the assay (120 minutes). For comparison BIBN4096BS blocks MMA dilation within 5 minutes of dosing but the effect has completely disappeared by 90 minutes. The magnitude of the block is comparable between BIBN4096BS and mu7E9.

Example 7: Chronic effect of anti-CGRP antagonist antibody G1 in a dural artery (closed cranial window) assay

[0218] The purpose of this experiment was to determine if the anti CGRP antibody could still block electrically stimulated MMA dilation 7 days after dosing. Preparation of the rats was identical to the above described acute experiment (Example 6) with the following exceptions. Rats were injected i.v. (10mg/kg, 3mg/kg or 1mg/kg G1) 7 days prior to creating the closed cranial window prep and stimulation. It was impossible to establish a baseline dilation response to electrical stimulation prior to dosing as in the acute experiment so the antibody groups were compared to dilation of the MMA in a vehicle (PBS, 0.01% Tween 20) dosed control group. After the rats were allowed to rest for no less than 45 minutes the dura was electrically stimulated at 30 minute intervals. Stimulations were at 2.5V, 5V, 10V, 15V and 20V, all at 10hz, 0.5 ms pulses for 30 seconds.

[0219] As shown in Figure 10 G1 at 10 mg/kg and 3 mg/kg significantly blocked MMA dilation evoked by electrical stimulation in the range of 10 to 20 volts. This data demonstrates that G1 can block electrically stimulated MMA dilation up to 7 days after dosing.

Example 8: Morphine withdrawal hot flush model

[0220] The morphine withdrawal rat model is an established rodent model for menopausal hot flush mechanisms (Sipe et al., Brain Res. 1028(2):191-202 (2004); Merchenthaler et al., Maturitas 30:307-316 (1998); Katovich et al., Brain Res. 494:85-94 (1989); Simpkins et al., Life Sciences 32:1957-1966 (1983)). Basically the rats are addicted to morphine by implanting morphine pellets under the skin. Upon addiction the animals are injected with naloxone (opioid antagonist) which sends them into withdrawal immediately. This withdrawal is accompanied by a skin temperature increase, a core body temperature decrease, an increase in heart rate and an increase in serum luteinizing hormone. These are all similar in magnitude and timing to what occurs in human hot flush (Simpkins et al., Life Sciences 32:1957-1966 (1983)).

Furthermore, if rats are treated with estradiol prior to inducing withdrawal, the symptoms of hot flush are reduced (Merchenthaler et al., Maturitas 30:307-316 (1998)). This is why the morphine withdrawal model is believed to mimic clinical hot flush.

[0221] Ovariectomized rats were ordered from Charles River Laboratories. Not less than 7 days post ovariectomy morphine dependency was created by implanting a morphine pellet (75 mg morphine base) subcutaneously. Two days later 2 more pellets were implanted. The following day rats were injected intravenously with either 10 mg/kg 4901 [**] or vehicle (PBS, 0.01% tween). Two days after the second pelleting the rats were anesthetized with ketamine (90 mg/kg) and lightly restrained. A surface temperature thermocouple was taped to the base of the tail and a rectal thermocouple is used to measure core temperature. Data was recorded using Chart software (ADInstruments). After recording 15 minutes of stable baseline temperature, naloxone (1mg/kg) was injected subcutaneously. Temperature was recorded continuously for the next 60 minutes. The results are shown in Figures 11A and 11B.

Deposit of Biological Material

[0222] The following materials have been deposited with the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, USA (ATCC):

Material	Antibody No.	ATCC Accession No.	Date of Deposit
pDb.CGRP.hFcGI	G1 heavy chain	PTA-6867	July 15, 2005
pEb.CGRP.hKGI	G1 light chain	PTA-6866	July 15, 2005

[0223] Vector pEb.CGRP.hKGI is a polynucleotide encoding the G1 light chain variable region and the light chain kappa constant region; and vector pDb.CGRP.hFcGI is a polynucleotide encoding the G1 heavy chain variable region and the heavy chain IgG2 constant region containing the following mutations: A330P331 to S330S331 (amino acid numbering with reference to the wildtype IgG2 sequence; see Eur. J. Immunol. (1999) 29:2613-2624).

[0224] These deposits were made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty). This assures maintenance of a viable culture of the deposit for 30 years from the date of deposit. The deposit will be made available by ATCC under the terms of the Budapest Treaty, and subject to an agreement between Rinat Neuroscience Corp. and ATCC, which assures permanent and unrestricted availability of the progeny of the culture of the deposit to the public upon issuance of the pertinent U.S. patent or upon laying open to the public of any U.S. or foreign patent application, whichever comes first, and assures availability of the progeny to one determined by the U.S. Commissioner of Patents and Trademarks to be entitled thereto according to 35 USC Section 122 and the Commissioner's rules pursuant thereto (including 37 CFR Section 1.14 with particular reference to 886 OG 638).

[0225] The assignee of the present application has agreed that if a culture of the materials on deposit should die or be lost or destroyed when cultivated under suitable conditions, the materials will be promptly replaced on notification with another of the same. Availability of the deposited material is not to be construed as a license to practice the invention in contravention of the rights granted under the authority of any government in accordance with its patent laws.

Antibody sequences

[0226]

G1 heavy chain variable region amino acid sequence (SEQ ID NO:1)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWISWVRQAPGKGLEWVAEIRSESDASATHYAEAVKG
RFTISRDNAKNSLYLQMNSLRAEDTAVYYCLAYFDYGLAIQNYWGQGTLVTVSS

G1 light chain variable region amino acid sequence (SEQ ID NO:2)
EIVLTQSPATLSLSPGERATLSCKASKRVTTYVSWYQQKPGQAPRLLIYGASNRYLGIPARFSGSGSGTDF
TLTISSLEPEDFAVYYCSQSYNYPYTFGQGTKLEIK

G1 CDR H1 (extended CDR) (SEQ ID NO:3)
GFTFSNYWIS

G1 CDR H2 (extended CDR) (SEQ ID NO:4)
EIRSESDASATHYAEAVKG

G1 CDR H3 (SEQ ID NO:5)
YFDYGLAIQNY

G1 CDR L1 (SEQ ID NO:6)
KASKRVTTYVS
G1 CDR L2 (SEQ ID NO:7)
GASNRYL

G1 CDR L3 (SEQ ID NO:8)
SQSYNYPYT

G1 heavy chain variable region nucleotide sequence (SEQ ID NO:9)
GAAGTTCAGCTGGTTGAATCCGGTGGTGGTCTGGTTCAGCCAGGTGGTTCCCTGCGTCTGTCCTGC
GCTGCTTCCGGTTTCACCTTCTCCAACTACTGGATCTCCTGGGTTCGTCAGGCTCCTGGTAAAGGTCT
GGAATGGGTTGCTGAAATCCGTTCCGAATCCGACGCGTCCGCTACCCATTACGCTGAAGCTGTTAAA
GGTCGTTTCACCATCTCCCGTGACAACGCTAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGTG
CTGAAGACACCGCTGTTTACTACTGCCTGGCTTACTTTGACTACGGTCTGGCTATCCAGAACTACTGG
GGTCAGGGTACCCTGGTTACCGTTTCCTCC

G1 light chain variable region nucleotide sequence (SEQ ID NO:10)
GAAATCGTTCTGACCCAGTCCCCGGCTACCCTGTCCCTGTCCCCAGGTGAACGTGCTACCCTGTCCTGC
AAAGCTTCCAAACGGGTTACCACCTACGTTTCCTGGTACCAGCAGAAACCCGGTCAGGCTCCTCGTCTG
CTGATCTACGGTGCTTCCAACCGTTACCTCGGTATCCCAGCTCGTTTCTCCGGTTCCGGTTCCGGTACCG

ACTTCACCCTGACCATCTCCTCCCTGGAACCCGAAGACTTCGCTGTTTACTACTGCAGTCAGTCCTACAA
CTACCCCTACACCTTCGGTCAGGGTACCAAACTGGAAATCAAA

G1 heavy chain full antibody amino acid sequence (including modified IgG2 as described herein) (SEQ ID NO: 11)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWISWVRQAPGKGLEWVAEIRSESDASATHYAEAVKGR
FTISRDNAKNSLYLQMNSLRAEDTAVYYCLAYFDYGLAIQNYWGQGTLVTVSSASTKGPSVFPLAPCSRS
TSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCNVDH
KPSNTKVDKTVERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWY
VDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPSSIEKTISKTKGQPREPQV

YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRW
QQGNVFSCSVMHEALHNHYTQKSLSLSPGK

G1 light chain full antibody amino acid sequence (SEQ ID NO:12)

EIVLTQSPATLSLSPGERATLSCKASKRVTTYVSWYQQKPGQAPRLLIYGASNRYLGIPARFSGSGSGTD
FTLTISSLEPEDFAVYYCSQSYNYPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP
REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC

G1 heavy chain full antibody nucleotide sequence (including modified IgG2 as described herein) (SEQ ID NO:13)

GAAGTTCAGCTGGTTGAATCCGGTGGTGGTCTGGTTCAGCCAGGTGGTTCCCTGCGTCTGTCCTGC
GCTGCTTCCGGTTTCACCTTCTCCAACTACTGGATCTCCTGGGTTCGTCAGGCTCCTGGTAAAGGTC
TGGAATGGGTTGCTGAAATCCGTTCCGAATCCGACGCGTCCGCTACCCATTACGCTGAAGCTGTTAA
AGGTCGTTTCACCATCTCCCGTGACAACGCTAAGAACTCCCTGTACCTGCAGATGAACTCCCTGCGT
GCTGAAGACACCGCTGTTTACTACTGCCTGGCTTACTTTGACTACGGTCTGGCTATCCAGAACTACTG
GGGTCAGGGTACCCTGGTTACCGTTTCCTCCGCCTCCACCAAGGGCCCATCTGTCTTCCCACTGGC
CCCATGCTCCCGCAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCC
AGAACCTGTGACCGTGTCCTGGAACTCTGGCGCTCTGACCAGCGGCGTGCACACCTTCCCAGCTGT
CCTGCAGTCCTCAGGTCTCTACTCCCTCAGCAGCGTGGTGACCGTGCCATCCAGCAACTTCGGCAC
CCAGACCTACACCTGCAACGTAGATCACAAGCCAAGCAACACCAAGGTCGACAAGACCGTGGAGAG
AAAGTGTTGTGTGGAGTGTCCACCTTGTCCAGCCCCTCCAGTGGCCGGACCATCCGTGTTCCTGTTC
CCTCCAAAGCCAAAGGACACCCTGATGATCTCCAGAACCCCAGAGGTGACCTGTGTGGTGGTGGAC
GTGTCCCACGAGGACCCAGAGGTGCAGTTCAACTGGTATGTGGACGGAGTGGAGGTGCACAACGCC
AAGACCAAGCCAAGAGAGGAGCAGTTCAACTCCACCTTCAGAGTGGTGAGCGTGCTGACCGTGGTG
CACCAGGACTGGCTGAACGGAAAGGAGTATAAGTGTAAGGTGTCCAACAAGGGACTGCCATCCAGC
ATCGAGAAGACCATCTCCAAGACCAAGGGACAGCCAAGAGAGCCACAGGTGTATACCCTGCCCCCA
TCCAGAGAGGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTGAAGGGATTCTATCCATCCG
ACATCGCCGTGGAGTGGGAGTCCAACGGACAGCCAGAGAACAACTATAAGACCACCCCTCCAATGC
TGGACTCCGACGGATCCTTCTTCCTGTATTCCAAGCTGACCGTGGACAAGTCCAGATGGCAGCAGGG
AAACGTGTTCTCTTGTTCCGTGATGCACGAGGCCCTGCACAACCACTATACCCAGAAGAGCCTGTCC
CTGTCTCCAGGAAAGTAA

G1 light chain full antibody nucleotide sequence (SEQ ID NO:14)

GAAATCGTTCTGACCCAGTCCCCGGCTACCCTGTCCCTGTCCCCAGGTGAACGTGCTACCCTGTCCT
GCAAAGCTTCCAAACGGGTTACCACCTACGTTTCCTGGTACCAGCAGAAACCCGGTCAGGCTCCTCG
TCTGCTGATCTACGGTGCTTCCAACCGTTACCTCGGTATCCCAGCTCGTTTCTCCGGTTCCGGTTCC
GGTACCGACTTCACCCTGACCATCTCCTCCCTGGAACCCGAAGACTTCGCTGTTTACTACTGCAGTC
AGTCCTACAACTACCCCTACACCTTCGGTCAGGGTACCAAACTGGAAATCAAACGCACTGTGGCTGC

ACCATCTGTCTTCATCTTCCCTCCATCTGATGAGCAGTTGAAATCCGGAACTGCCTCTGTTGTGTGCC

TGCTGAATAACTTCTATCCGCGCGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCCG
GTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCC
TGACCCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCC
TGAGTTCTCCAGTCACAAAGAGCTTCAACCGCGGTGAGTGCTAA

Amino acid sequence comparison of human and rat CGRP (human α-CGRP (SEQ ID NO:15); human β-CGRP (SEQ ID NO:43); rat α-CGRP (SEQ ID NO:41); and rat β-CGRP (SEQ ID NO:44)): NH_2-ACDTATCVTHRLAGLLSRSGGVVKNNFVPTNVGSKAF-$CONH_2$ (human α-CGRP) NH_2-ACNTATCVTHRLAGLLSRSGGMVKSNFVPTNVGSKAF-$CONH_2$ (human β-CGRP) NH_2-SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSEAF-$CONH_2$ (rat α-CGRP) NH_2-SCNTATCVTHRLAGLLSRSGGVVKDNFVPTNVGSKAF-$CONH_2$ (rat β-CGRP)

Numbered embodiments

[0227]

1. 1. An antibody with a binding affinity (K_D) to human α-CGRP of 50 nM or less as measured by surface plasmon resonance at 37 °C.
2. 2. The antibody according to numbered embodiment 1, comprising a V_H domain that is at least 90% identical in amino acid sequence to SEQ ID NO: 1.
3. 3. The antibody according to numbered embodiment 2, wherein the amino acid residue at position 99 of SEQIDNO: 1 is L or is substituted by A, N, S, T, V or R, and wherein the amino acid residue at position 100 of SEQIDNO: 1 is A, or is substituted by L, R, S, V, Y, C G, T, K or P.
4. 4. The antibody according to numbered embodiment 1, comprising a V_L domain that is at least 90% identical in amino acid sequence to SEQ ID NO: 2.
5. 5. The antibody according to numbered embodiment 1, comprising at least one CDR selected from the group consisting of:
    1. a. CDR H1 as set forth in SEQ ID NO: 3;
    2. b. CDR H2 as set forth in SEQ ID NO: 4;
    3. c. CDR H3 as set forth in SEQ ID NO: 5;
    4. d. CDR L1 as set forth in SEQ ID NO: 6;
    5. e. CDR L2 as set forth in SEQ ID NO: 7;
    6. f. CDR L3 as set forth in SEQ ID NO: 8; and
    7. g. variants of L1, L2 and H2 as shown in Table 6.
6. 6. The antibody according to numbered embodiment 1, comprising:
    1. a. a V_H CDR3 as set forth in SEQ ID NO: 5, or a sequence that differs from SEQ

ID NO: 5 by 1 or 2 conservative amino acid substitutions; and

2. b. a V_L CDR3 as set forth in SEQ ID NO: 8, or a sequence that differs from SEQ ID NO: 8 by 1 or 2 conservative amino acid substitutions.

7. 7. An antibody comprising a V_H domain that is at least 90% identical in amino acid sequence to SEQ ID NO: 1 and a V_L domain that is at least 90% identical in amino acid sequence to SEQ ID NO:2.
8. 8. The antibody of numbered embodiment 7, wherein the antibody is an IgG, an IgM, an IgE, an IgA, or an IgD molecule, or is derived therefrom.
9. 9. The antibody according to numbered embodiment 7, comprising a heavy chain produced by the expression vector with ATCC Accession No. PTA-6867.
10. 10. The antibody according to numbered embodiment 7, comprising a light chain produced by the expression vector with ATCC Accession No. PTA-6866.
11. 11. A pharmaceutical composition comprising the antibody according to numbered embodiment 1 and a pharmaceutically acceptable excipient.
12. 12. A method for preventing or treating at least one vasomotor symptom in an individual, comprising administering to the individual an effective amount of an anti-CGRP antagonist antibody.
13. 13. The method according to numbered embodiment 12, where said vasomotor symptom is a migraine with or without aura, hemiplegic migraine, cluster headache, migrainous neuralgia, chronic headache, or tension headache.
14. 14. The method according to numbered embodiment 12, where said vasomotor symptom is hot flush.
15. 15. The method of numbered embodiment 12, where the anti-CGRP antagonist antibody is any of the antibodies described in numbered embodiments 1 to 10.
16. 16. The method of numbered embodiment 12, where the anti-GCRP antagonist antibody is the antibody produced by the expression vectors with ATCC Accession Nos. PTA-6867 and PTA-6866.

SEQUENCE LISTING

[0228]

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<141> 2005-11-14

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Trp Ile Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
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Ala Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser
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Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
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35 40 45

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50 55 60

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cctggtaaag gtctggaatg ggttgctgaa atccgttccg aatccgacgc gtccgctacc 180

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20 25 30

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35 40 45

Ala Glu Ile Arg Ser Glu Ser Asp Ala Ser Ala Thr His Tyr Ala Glu
50 55 60

Ala Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser
65 70 75 80

Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr
85 90 95

Tyr Cys Leu Ala Tyr Phe Asp Tyr Gly Leu Ala Ile Gln Asn Tyr Trp
100 105 110

Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
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Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr
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His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys
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Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser
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Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg
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Val Ser Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile
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Tyr Gly Ala Ser Asn Arg Tyr Leu Gly Ile Pro Ala Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Glu Pro
65 70 75 80

Glu Asp Phe Ala Val Tyr Tyr Cys Ser Gln Ser Tyr Asn Tyr Pro Tyr
85 90 95

Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
100 105 110

Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
115 120 125

Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
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<210> 35
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Gly Ser Lys Ala
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Gly Ser Glu Ala Phe
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Gly Ser Lys Ala Phe
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Gly Ser Lys Ala Phe
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20 25 30

Phe Thr Asp Lys Asp Lys Asp Asn Val Ala Pro Arg Ser Lys Ile Ser
35 40 45

Pro Gln Gly Tyr
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REFERENCES CITED IN THE DESCRIPTION

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Patentkrav

1. Anti-CGRP-antagonistantistof til anvendelse i forebyggelsen eller behandling af klynge-hovedpine.

2. Anti-CGRP-antagonistantistoffet til anvendelse ifølge krav 1, hvor antistoffet er et humant antistof eller et humaniseret antistof.

3. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet har en bindingsaffinitet (K_D) til human α-CGRP på 50 nM eller derunder som målt ved overfladeplasmon-resonans ved 37°C.

4. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet binder (a) det C-terminale fragment med aminosyrerne 25-37 af CGRP eller (b) en C-terminal epitop indenfor aminosyrerne 25-37 af CGRP.

5. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet har en IgG tung kæde konstant region.

6. Anti-CGRP-antagonistantistoffet til anvendelse ifølge krav 5, hvor antistoffet har en IgG1, IgG2, IgG3 eller IgG4 tung kæde konstant region.

7. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet er: (a) et antistof med en CDR H1 som vist i SEQ ID NO: 3; en CDR H2 som vist i SEQ ID NO: 4; en CDR H3 som vist i SEQ ID NO: 5; en CDR L1 som vist i SEQ ID NO: 6; en CDR L2 som vist i SEQ ID NO: 7; og en CDR L3 som vist i SEQ ID NO: 8; eller (b) en variant af et antistof af (a) som vist i tabel 6.

8. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet omfatter et V_H-domæne der er mindst 90% identisk i aminosyresekvensen til SEQ ID NO: 1 og et V_L-domæne der er mindst

90% identisk i aminosyresekvensen til SEQ ID NO: 2; eksempelvis, hvor antistoffet omfatter et V_H-domæne af SEQ ID NO: 1 og et V_L-domæne af SEQ ID NO: 2.

9. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet omfatter en let kæde fremstillet af en ekspressionsvektor med ATCC Accession No. PTA-6866 og/eller en tung kæde fremstillet af ekspressionsvektoren med ATCC Accession No. PTA-6867.

10. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet blokerer eller formindsker CGRP-receptor-aktivering (herunder cAMP-aktivering).

11. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet administreres ved injektion; fx intraperitonealt, intravenøst, subkutant eller intramuskulært.

12. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet administreres 1 til 4 gange om ugen.

13. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet administreres ved en daglig dosering på fra 3 µg/kg til 100 mg/kg.

14. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet administreres ved ca. 2 mg/kg.

15. Anti-CGRP-antagonistantistoffet til anvendelse ifølge et hvilket som helst af de foregående krav, hvor antistoffet administreres til et menneske.

16. Anvendelsen af et anti-CGRP-antagonistantistof i fremstillingen af et medikament til forebyggelse eller behandling af klynge-hovedpine.

Figure 1

Fab	K_D (nM) 1-37 (WT)	K_D (nM) 19-37[a]	*K_D (nM) 25-37[a]*	K_D (mutant/parent) F27A	V28A	P29A	T30A	N31A	V32A	G33A	S34A	K35A	F37A
7E9	1.0	1.1±0.8	*0.14±0.05*	**1.0**	**1.0**	**26**	**7**	**9**	**41**	**1256**	**69**	**4**	**3598**
8B6	1.1	1.5±1.2	*0.45±0.08*	**1.0**	**1.0**	**9**	**2.2**	**3**	**5**	**496**	**26**	**3**	**2527**
10A8	2.1	2.4±1.4	*1.0±0.2*	**1.0**	**1.0**	**9**	**4**	**4**	**11**	**36**	**82**	**13**	**2152**
7D11	4.4	10±7	*3.4±0.4*	**1.1**	**1.0**	**7**	**4**	**5**	**5**	**86**	**18**	**1.4**	**420**
6H2	9.3	7.8±0.2	*8.5±0.5*	**0.9**	**1.0**	**1.0**	**0.8**	**4**	**11**	**14**	**0.5**	**1.0**	
4901	60.5	52±12	*296±115*	**0.8**	**0.8**	**0.2**	**0.2**	**0.3**	**0.9**	**1.3**	**0.8**	**0.3**	
14E10	79.7	91±3	*117.4±0.7*	**0.8**	**0.8**	**11**	**3**	**18**	**2**	**1**	**3**	**0.4[b]**	
9B8	84.7	76±20	*96±28*	**0.8**	**0.8**	**0.6**	**0.6**	**0.7**	**0.6**	**1.3**	**4**	**0.4[b]**	
13C2	94.4	86±13	*137±5*	**0.7**	**0.7**	**0.5**	**0.4**	**0.6**	**0.2**	**0.9**	**1.1**	**0.4[b]**	
14A9	148.4	219±114	*246±20*	**0.8**	**0.7**	**0.7**	**0.5**	**0.8**	**0.7**	**1.6**	**1.3**	**6**	
6D5	209.9	207±26	*378±22*	**0.8**	**0.7**	**0.5**	**0.4**	**0.6**	**0.5**	**3**	**1.1**	**5**	
1C5	296.4	223±51	*430±173*	**0.8**	**0.8**	**0.6**	**0.4**	**0.6**	**0.6**	**1.1**	**1.1**	**5**	

Figure 2A

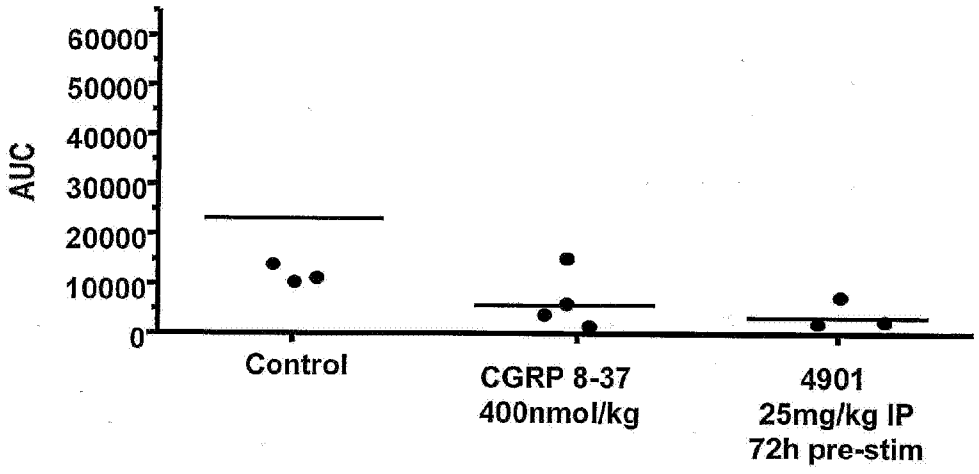

AUC
60000
50000
40000
30000
20000
10000
0
Control
CGRP 8-37
400nmol/kg
4901
25mg/kg IP
72h pre-stim

Figure 2B

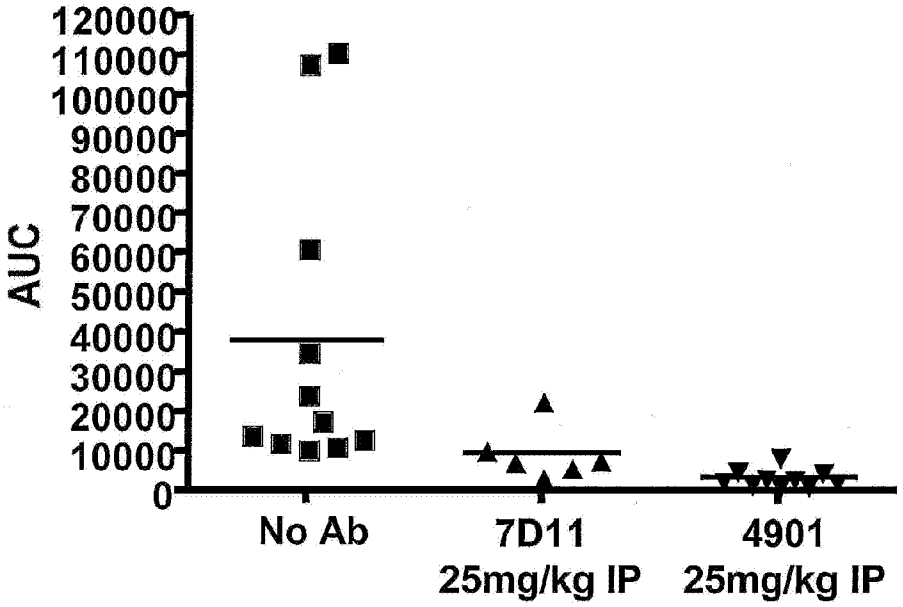

120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
AUC
No Ab
7D11
25mg/kg IP
4901
25mg/kg IP

Figure 3

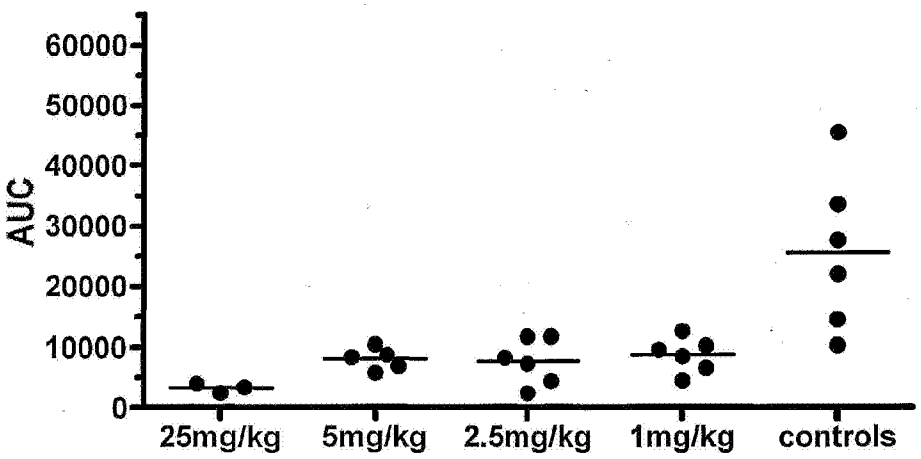

AUC
60000
50000
40000
30000
20000
10000
0
25mg/kg
5mg/kg
2.5mg/kg
1mg/kg
controls

Figure 4A

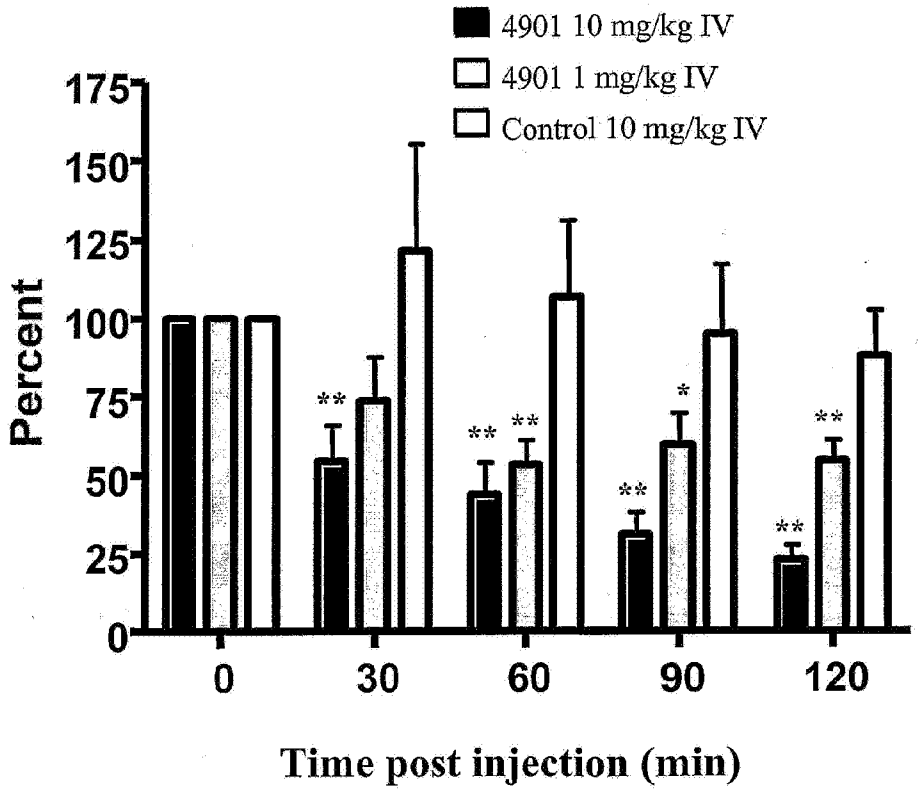

4901 10 mg/kg IV
4901 1 mg/kg IV
Control 10 mg/kg IV
Percent
175
150
125
100
75
50
25
0
**
**
**
**
*
**
**
0
30
60
90
120
Time post injection (min)

Figure 4B

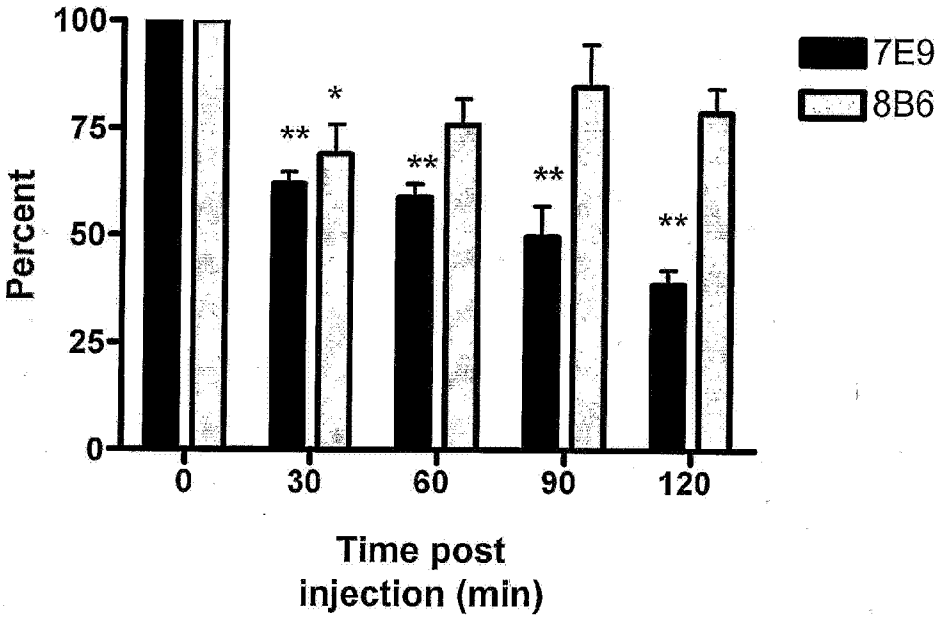

100
75
50
25
0
Percent
**
*
**
**
**
0
30
60
90
120
Time post
injection (min)
7E9
8B6

Figure 5

Bold=Kabat CDR
Underline=Chothia CDR

G1 Heavy chain

```
                                                                    H1
1         5         10        15        20        25        30
E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
                                                  H2
31        35        40        45        50        55        60
N Y W I S W V R Q A P G K G L E W V A E I R S E S D A S A T

61        65        70        75        80        85        90
H Y A E A V K G R F T I S R D N A K N S L Y L Q M N S L R A
                                H3
91        95        100       105       110       115       120
E D T A V Y Y C L A Y F D Y G L A I Q N Y W G Q G T L V T V

121 122
S S
```

G1 Light chain

```
                                                                    L1
1         5         10        15        20        25        30
E I V L T Q S P A T L S L S P G E R A T L S C K A S K R V T
                                                  L2
31        35        40        45        50        55        60
T Y V S W Y Q Q K P G Q A P R L L I Y G A S N R Y L G I P A

61        65        70        75        80        85        90
R F S G S G S G T D F T L T I S S L E P E D F A V Y Y C S Q
   L3
91        95        100       105   107
S Y N Y P Y T F G Q G T K L E I K
```

Figure 6

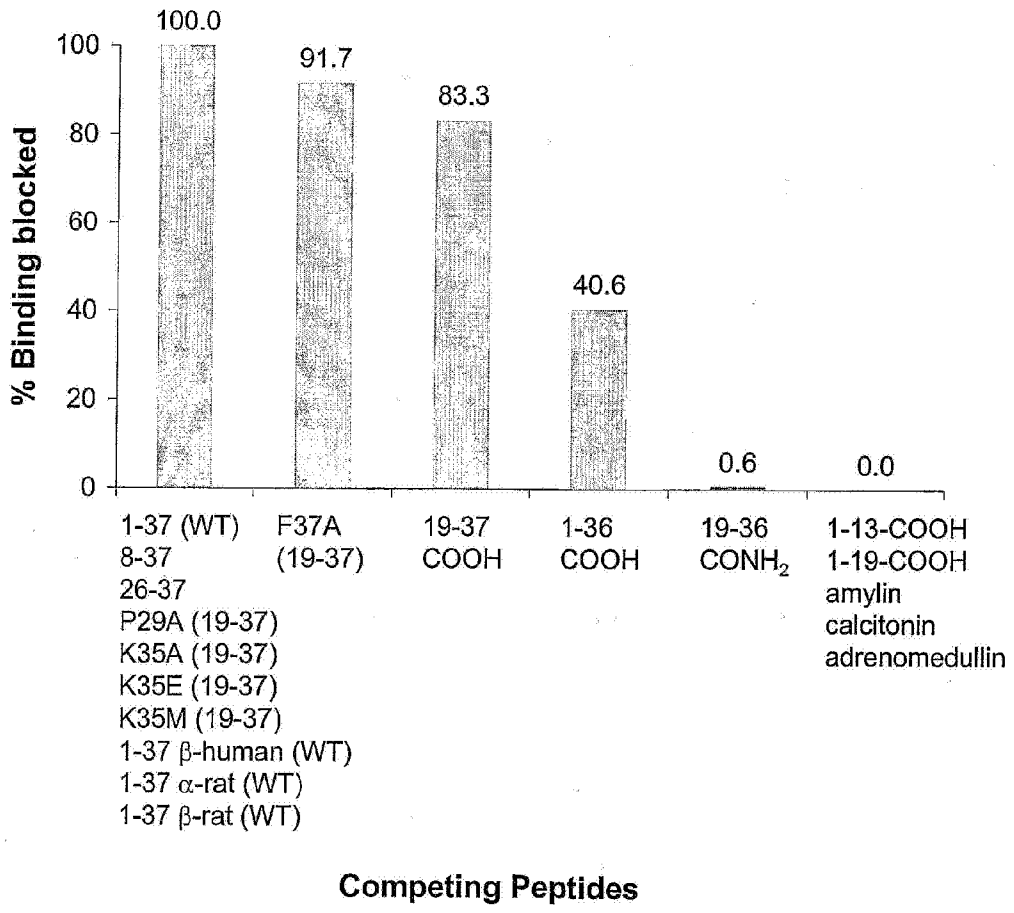

% Binding blocked
100
80
60
40
20
0
100.0
91.7
83.3
40.6
0.6
0.0
1-37 (WT)
8-37
26-37
P29A (19-37)
K35A (19-37)
K35E (19-37)
K35M (19-37)
1-37 β-human (WT)
1-37 α-rat (WT)
1-37 β-rat (WT)
F37A
(19-37)
19-37
COOH
1-36
COOH
19-36
CONH2
1-13-COOH
1-19-COOH
amylin
calcitonin
adrenomedullin
Competing Peptides

Figure 7

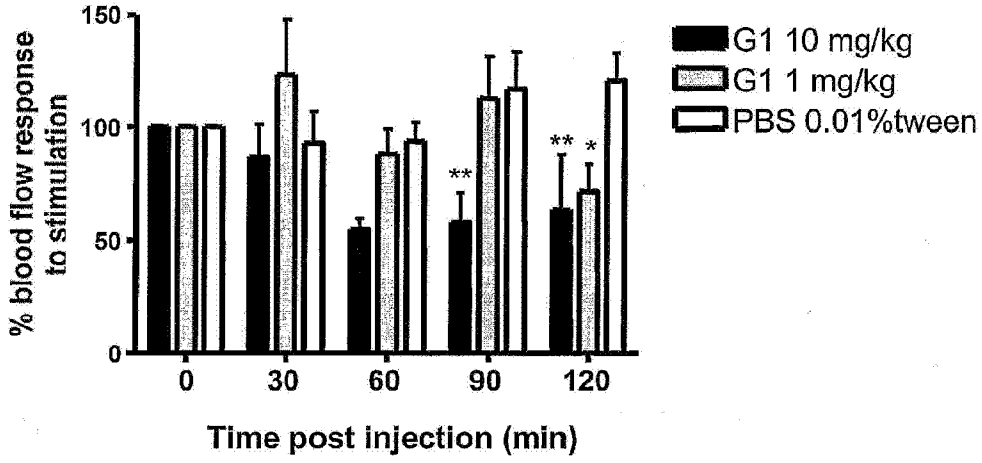

150
100
50
0
% blood flow response to stimulation
0
30
60
90
120
Time post injection (min)
**
**
*
G1 10 mg/kg
G1 1 mg/kg
PBS 0.01%tween

Figure 8A

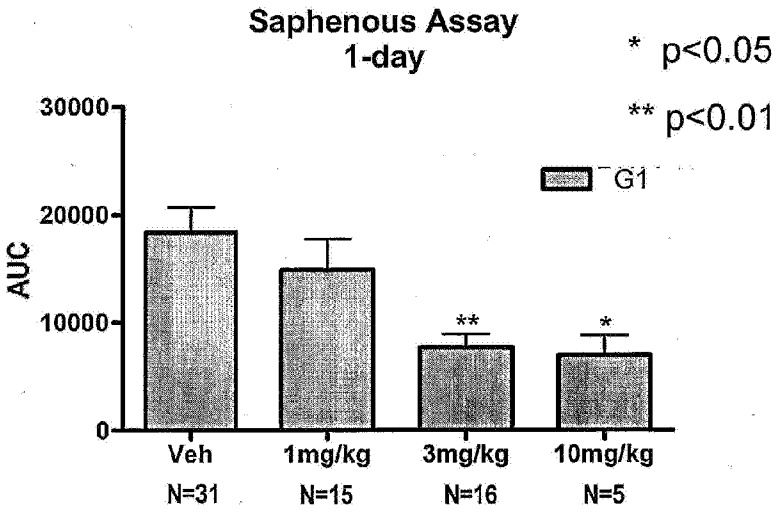

Saphenous Assay
1-day
* p<0.05
** p<0.01
G1
AUC
30000
20000
10000
0
**
*
Veh
N=31
1mg/kg
N=15
3mg/kg
N=16
10mg/kg
N=5

Figure 8B

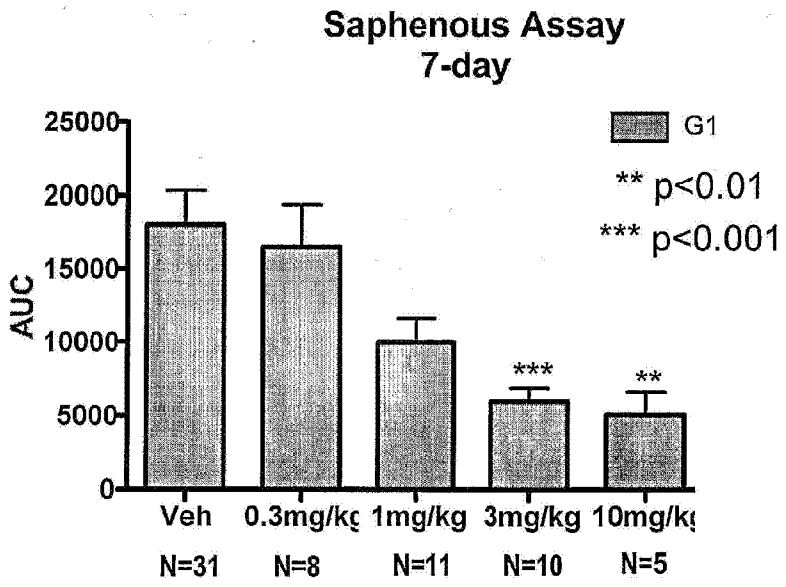

Saphenous Assay
7-day
G1
** p<0.01
*** p<0.001
AUC
25000
20000
15000
10000
5000
0
***
**
Veh
0.3mg/kg
1mg/kg
3mg/kg
10mg/kg
N=31
N=8
N=11
N=10
N=5

Figure 8C

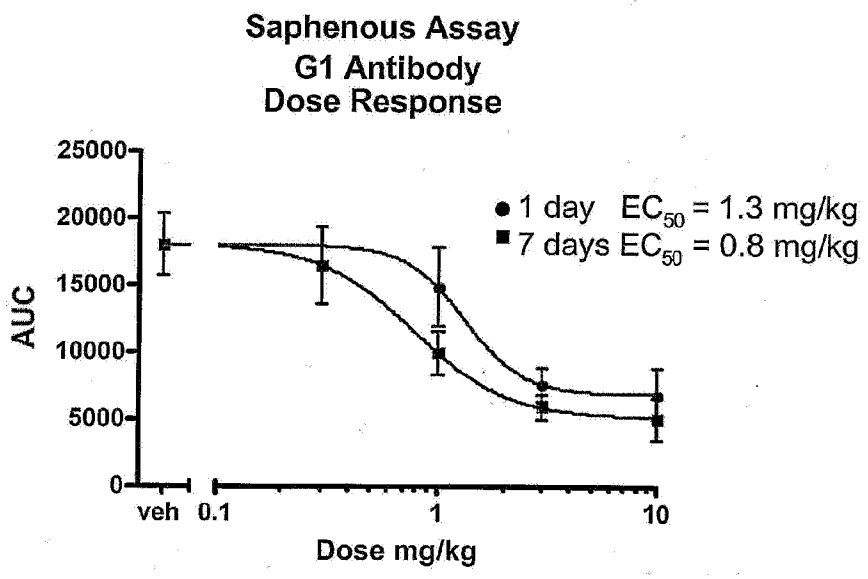

Saphenous Assay
G1 Antibody
Dose Response
25000
20000
15000
10000
5000
0
AUC
● 1 day EC50 = 1.3 mg/kg
■ 7 days EC50 = 0.8 mg/kg
veh
0.1
1
10
Dose mg/kg

Figure 9

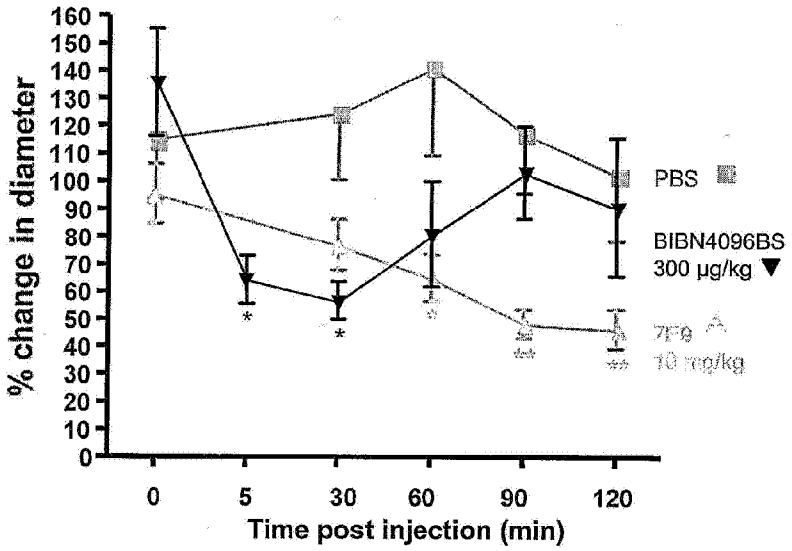

% change in diameter
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
0
5
30
60
90
120
Time post injection (min)
PBS
BIBN4096BS
300 µg/kg
10 mg/kg

Figure 10

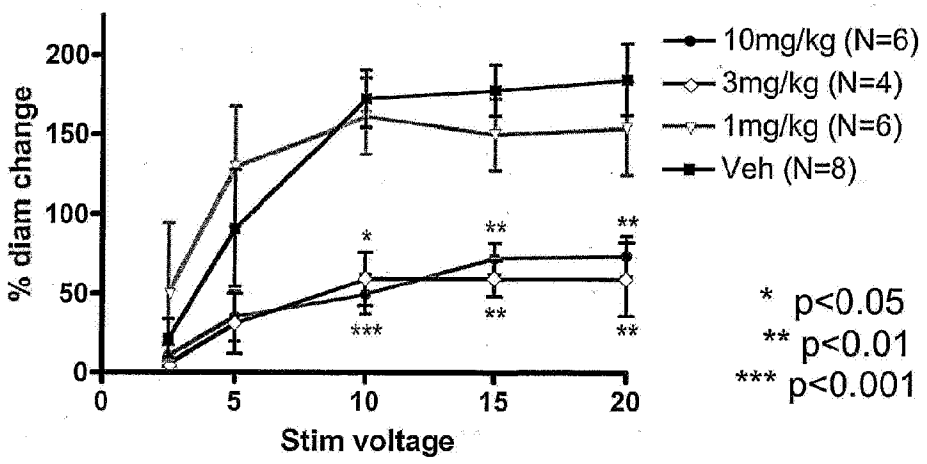

% diam change
200
150
100
50
0
0
5
10
15
20
Stim voltage
10mg/kg (N=6)
3mg/kg (N=4)
1mg/kg (N=6)
Veh (N=8)
*
**
***
* p<0.05
** p<0.01
*** p<0.001

Figure 11A

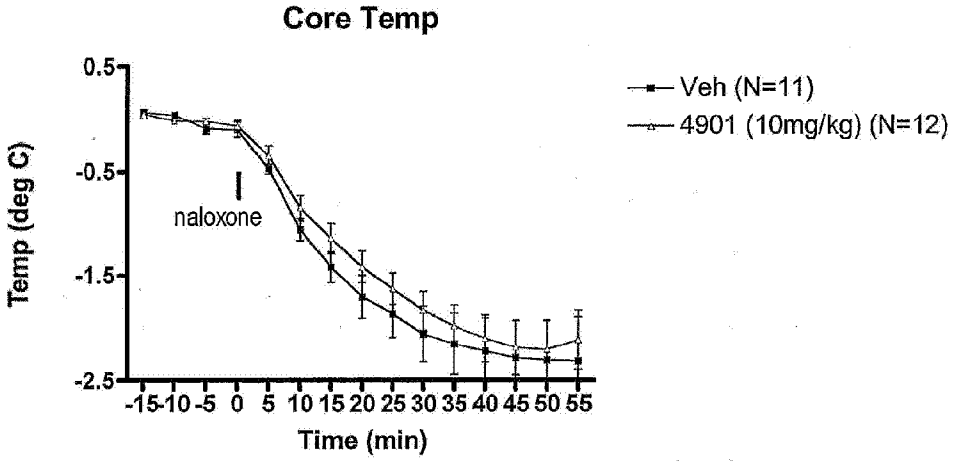
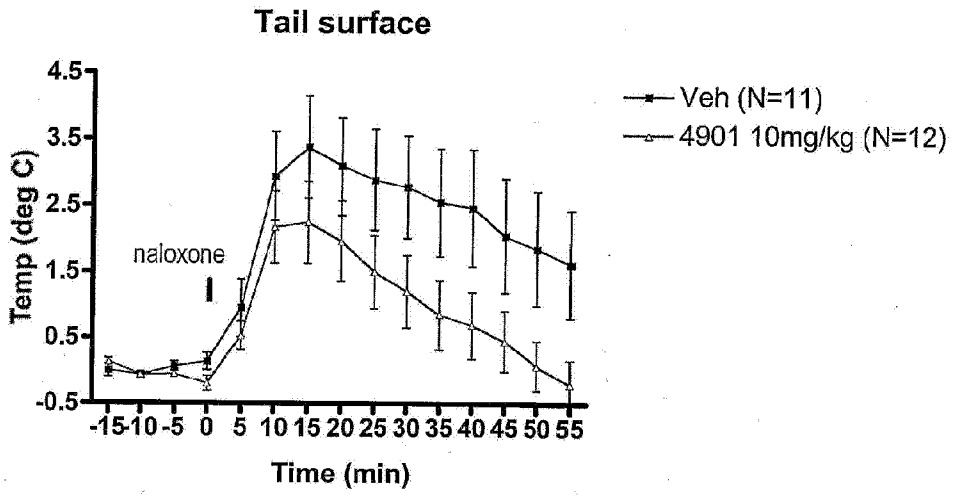

Core Temp
0.5
-0.5
-1.5
-2.5
Temp (deg C)
naloxone
-15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)
Veh (N=11)
4901 (10mg/kg) (N=12)

Figure 11B


Tail surface
Temp (deg C)
4.5
3.5
2.5
1.5
0.5
-0.5
naloxone
-15 -10 -5 0 5 10 15 20 25 30 35 40 45 50 55
Time (min)
Veh (N=11)
4901 10mg/kg (N=12)