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(71) Demandeur/Applicant:
ELI LILLY AND COMPANY, US
(72) Inventeurs/Inventors:
BEIDLER, CATHERINE BRAUTIGAM, US;
JOHNSON, MICHAEL PARVIN, US;
PATEL, CHETANKUMAR NATVARLAL, US
(74) Agent: GOWLING WLG (CANADA) LLP

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(57) **Abrégé/Abstract:**

Antibodies to human pituitary adenylate cyclase-activating peptide, compositions comprising such antibodies, and methods of using such antibodies for the treatment of pain including headache and / or migraine.

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(71) Applicant: **ELI LILLY AND COMPANY** [US/US]; Lilly
Corporate Center, Indianapolis, Indiana 46285 (US).

(72) Inventors: **BEIDLER, Catherine Brautigam**; c/o Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US). **JOHNSON, Michael Parvin**; c/o Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US). **PATEL, Chetankumar Natvarlal**; c/o Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

(74) Agent: **MARKS, Duane C.** et al.; Eli Lilly and Company, P.O. Box 6288, Indianapolis, Indiana 46206-6288 (US).

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(54) Title: ANTI-PACAP ANTIBODY

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ANTI-PACAP ANTIBODY

The present invention is in the field of medicine. Particularly, the present invention relates to antibodies to pituitary adenylate cyclase-activating peptide (PACAP), compositions comprising such anti-PACAP antibodies, and methods of using such antibodies for the treatment of pain including primary headaches (including trigeminal autonomic cephalalgias), secondary headaches and migraines (including chronic migraine).

Pituitary adenylate cyclase-activating peptide (PACAP) is a neuropeptide distributed throughout the nervous system including the trigeminovascular system, trigeminal ganglia, spinal cord, hypothalamus, and pituitary. PACAP exists in at least two α -amidated active forms: PACAP38 (SEQ ID NO: 13), which comprises 38 amino acids and is the more prevalent active form, typically representing up to 90% of PACAP in mammalian tissue; and PACAP27 (SEQ ID NO: 14) which comprises the same 27 N-terminal amino acids as PACAP38. PACAP is believed to play roles in neuroprotection, neuromodulation, neurogenic inflammation and nociception and in causing pain, including headaches and migraines.

Headaches and migraines are estimated to impact 37 million patients a year, with more than two-thirds being untreated. Primary headache(s) is classified as headaches not resultant from a different or separate disease or disorder, while secondary headache(s) is classified as headaches resultant from a different or separate underlying cause (e.g., trauma, illness, or other disorder). Trigeminal autonomic cephalalgias ("TACs") are classified as primary headaches that include episodic and chronic cluster headache, paroxysmal hemicrania, hemicranias continua, and unilateral neuralgiform headache attacks. Migraine(s) refers to migraines "without aura" (formerly termed "common migraines") and "with aura" (formerly termed "classical migraines"). Chronic migraine(s) is classified as 15 or more headache days per month with at least eight of which are migraines. When migraine prevalence is two or more episodes per month, or when migraines significantly interfere with a patient's daily routine and/or acute medications are ineffective, physicians are encouraged to consider preventative treatment options. However, to date treatment options for migraine prophylaxis are often ineffective and current preventative and acute treatment options (e.g., antihypertensives, anticonvulsants, antidepressants) have low efficacy and associated disabling side effects.

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The structure of PACAP is well-known in the art (see, for example, A. Miyata, A. et al., Biochem Biophys Res Commun 170: 643-648 (1990)) as are anti-PACAP antibodies. For example, U.S. Patent No. 5,486,472A, International Patent Application Publication No. WO/2012/106407 A3, and U.S. Patent Application Publication No. 2016-304604 all disclose various anti-PACAP antibodies and potential uses thereof. However, to date, no antibody targeting PACAP has been approved for therapeutic use. Thus, there remains a need for alternative anti-PACAP antibodies. In particular, there remains a need for alternative anti-PACAP antibodies that neutralize PACAP with high potency, provide a sustained duration of action, and are capable of treating pain including primary and secondary headaches and migraines including chronic migraine. As with all therapeutic treatments, safety and toxicity remain a limitation and alternative anti-PACAP antibodies must not be attendant on unacceptable immunogenicity. There, thus, remains a need for alternative anti-PACAP antibodies which present a reduced risk of immunogenicity in humans. Such anti-PACAP antibodies will preferably also possess good physical-chemical properties to facilitate development, manufacturing, and formulation.

The present invention provides an antibody that binds human PACAP and which comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR comprises the complementarity determining regions (CDRs) HCDR1, HCDR2 and HCDR3 and the LCVR comprises CDRs LCDR1, LCDR2 and LCDR3, wherein the amino acid sequence of HCDR1 is SEQ ID NO.3, the amino acid sequence of HCDR2 is SEQ ID NO.4, the amino acid sequence of HCDR3 is SEQ ID NO.5, the amino acid sequence of LCDR1 is SEQ ID NO.6, the amino acid sequence of LCDR2 is SEQ ID NO.7, and the amino acid sequence of LCDR3 is SEQ ID NO.8. In a particular embodiment HCDR2 comprises aspartic acid at residue 13, HCDR3 comprises asparagine at residue 8, LCDR1 comprises serine at residue 7, and LCDR2 comprises leucine at residue 7. In another particular embodiment, HCDR2 comprises alanine at residue 13, HCDR3 comprises threonine at residue 8, LCDR1 comprises tryptophan at residue 7, and LCDR2 comprises phenylalanine at residue 7. In a further particular embodiment, HCDR2 comprises glutamic acid at residue 13, HCDR3 comprises threonine at residue 8, LCDR1 comprises tryptophan at residue 7, and LCDR2 comprises phenylalanine at residue 7. In an even further particular embodiment, HCDR2 comprises

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glutamine at residue 13, HCDR3 comprises threonine at residue 8, LCDR1 comprises tryptophan at residue 7, and LCDR2 comprises phenylalanine at residue 7.

Embodiments of the present invention provide an antibody that binds human PACAP, comprising a HCVR and a LCVR, wherein the amino acid sequence of the

5 HCVR is SEQ ID NO.9 and the amino acid sequence of the LCVR is SEQ ID NO. 10. In particular embodiments, HCVR comprises aspartic acid at residue 62 and asparagine at residue 104, and LCVR comprises serine at residue 30 and leucine at residue 55. In other particular embodiments, HCVR comprises alanine at residue 62 and threonine at residue 104, and LCVR comprises tryptophan at residue 30 and phenylalanine at residue 55. In
10 some embodiments, HCVR comprises glutamic acid at residue 62 and threonine at residue 104, and LCVR comprises tryptophan at residue 30 and phenylalanine at residue 55. In some embodiments, HCVR comprises glutamine at residue 62 and threonine at residue 104, and LCVR comprises tryptophan at residue 30 and phenylalanine at residue 55.

15 In further embodiments, the present invention provides an antibody that binds human PACAP, comprising a heavy chain (HC) and a light chain (LC), wherein the amino acid sequence of the HC is SEQ ID NO.1 and the amino acid sequence of the LC is SEQ ID NO.2. In particular embodiments, HC comprises aspartic acid at residue 62, asparagine at residue 104, proline at residue 231, alanine at residue 237 and alanine at
20 residue 238, and LC comprises serine at residue 30 and leucine at residue 55. In other particular embodiments, HC comprises alanine at residue 62, threonine at residue 104, proline at residue 231, alanine at residue 237 and alanine at residue 238, and LC comprises tryptophan at residue 30 and phenylalanine at residue 55. In other particular
25 embodiments HC comprises glutamic acid at residue 62, threonine at residue 104, proline at residue 231, alanine at residue 237 and alanine at residue 238, and LC comprises tryptophan at residue 30 and phenylalanine at residue 55. In some particular
embodiments HC comprises glutamine at residue 62, threonine at residue 104, proline at residue 231, alanine at residue 237 and alanine at residue 238, and LC comprises
tryptophan at residue 30 and phenylalanine at residue 55.

30 The present invention further provides pharmaceutical compositions comprising an antibody of the present invention and one or more pharmaceutically acceptable carriers, diluents or excipients. Further, the present invention provides a method of

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treating pain such as headaches, including primary and secondary headache comprising administering to a patient in need thereof a pharmaceutical composition of the present invention. In an even further embodiment, the primary headache is a TAC. In an even further embodiment, the present invention provides a method of treating migraines

5 comprising administering to a patient in need thereof a pharmaceutical composition of the present invention. In some such embodiments, the migraine is chronic migraine. Further, the present invention provides a method of treating mast cell degranulation related pain comprising administering to a patient in need thereof an antibody or pharmaceutical composition of the present invention. According to some such embodiments mast cell
10 degranulation related pain is one of primary or secondary headaches and migraine.

In addition, the present invention provides a method of treating pain such as primary headache, secondary headache and/or migraine comprising administering to a patient in need thereof an effective amount of an antibody of the present invention. According to some embodiments, the primary headache is a TAC. According to some
15 embodiments, the migraine is chronic migraine.

The present invention also provides an antibody of the present invention for use in therapy. More particularly, the present invention provides an antibody of the present invention for use in treatment of pain. In particular embodiments, the present invention provides an antibody of the present invention for use in treatment of primary headache,
20 secondary headache and/or migraine.

Further, the present invention provides the use of an antibody of the present invention in the manufacture of a medicament for the treatment of pain such as primary headache, secondary headache and/or migraine. In even more particular embodiments, the primary headache is a TAC. In some particular embodiments, the migraine is chronic
25 migraine.

The present invention also relates to nucleic acid molecules and expression vectors encoding the antibodies of the present invention. In an embodiment, the present invention provides a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO.1, wherein residue 62 is
30 aspartic acid, residue 104 is asparagine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine. In some embodiments, residue 62 is alanine, residue 104 is threonine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine. In

some embodiments, residue 62 is glutamic acid, residue 104 is threonine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine. In further embodiments, residue 62 is glutamine, residue 104 is threonine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine

5 Embodiments of the present invention also provide a DNA molecule comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO.2, wherein residue 30 is serine and residue 55 is leucine. In some embodiments, residue 30 is tryptophan and residue 55 is phenylalanine.

 In some embodiments, the DNA molecule of the present molecule comprises a
10 polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO.1 wherein residue 62 is aspartic acid, residue 104 is asparagine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine, and comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO. 2 wherein residue 30 is serine and residue 55 is leucine. In a particular
15 embodiment the DNA molecule of the present molecule comprises a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO.1 wherein residue 62 is alanine, residue 104 is threonine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine, and comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO.2 wherein residue 30 is
20 tryptophan and residue 55 is phenylalanine. In a particular embodiment the DNA molecule of the present molecule comprises a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO.1 wherein residue 62 is glutamic acid, residue 104 is threonine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine, and comprising a polynucleotide sequence encoding a polypeptide
25 having the amino acid sequence of SEQ ID NO.2 wherein residue 30 is tryptophan and residue 55 is phenylalanine. In another particular embodiment the DNA molecule of the present molecule comprises a polynucleotide sequence encoding a polypeptide having an amino acid sequence of SEQ ID NO.1 wherein residue 62 is glutamine, residue 104 is threonine, residue 231 is proline, residue 237 is alanine and residue 238 is alanine, and
30 comprising a polynucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO. 2 wherein residue 30 is tryptophan and residue 55 is phenylalanine. Further, the present invention provides an antibody prepared according to

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a process, wherein said process comprises cultivating a host cell comprising a polynucleotide sequence of the present invention, under conditions such that the antibody is expressed, and recovering from said host cell an antibody of the present invention.

As used herein, an “antibody” is an immunoglobulin molecule comprising 2 HCs and 2 LCs interconnected by disulfide bonds. The amino terminal portion of each LC and HC includes a variable region of about 100-120 amino acids primarily responsible for antigen recognition via the CDRs contained therein. The CDRs are interspersed with regions that are more conserved, termed framework regions (“FR”). Each LCVR and HCVR is composed of 3 CDRs and 4 FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The 3 CDRs of the LC are referred to as “LCDR1, LCDR2, and LCDR3,” and the 3 CDRs of the HC are referred to as “HCDR1, HCDR2, and HCDR3.” The CDRs contain most of the residues which form specific interactions with the antigen. The functional ability of an antibody to bind a particular antigen is largely influenced by the six CDRs.

Assignment of amino acids to CDR domains within the LCVR and HCVR regions of the antibodies of the present invention is based on the well-known Kabat numbering convention (Kabat, et al., *Ann. NY Acad. Sci.* 190:382-93 (1971); Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242 (1991)), and North numbering convention (North et al., *A New Clustering of Antibody CDR Loop Conformations*, *Journal of Molecular Biology*, 406:228-256 (2011)).

LCs are classified as kappa or lambda, which are each characterized by a particular constant region as known in the art. The antibodies of the present invention include kappa LCs. HCs are classified as gamma, mu, alpha, delta, or epsilon, and define the isotype of an antibody as IgG, IgM, IgA, IgD, or IgE, respectively. The antibodies of the present invention include IgG HCs. IgG antibodies can be further divided into subclasses, e.g., IgG1, IgG2, IgG3, IgG4. In a particular embodiment, the antibodies of the present invention are IgG4. The carboxy-terminal portion of each HC defines a constant region primarily responsible for effector function. In a particular embodiment, the antibodies of the present invention have one or more modifications in the constant region of each HC that reduces effector function. In a more particular embodiment, the antibodies of the present invention are IgG4 and have modifications in the constant region

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of both HCs that reduce effector function including the amino acid alanine at both residues 237 and 238 (residue numbering is linear and based on the exemplified HC of SEQ ID NO.1). In an even more particular embodiment, the antibodies of the present invention are IgG4 and have modifications in the constant region of both HCs that reduce effector function including the amino acid alanine at both residues 237 and 238 and have further modifications in the constant region of both HCs promoting stability including the amino acid proline at residue 231 (residue numbering is linear and based on the exemplified HC of SEQ ID NO.1).

The antibodies of the present invention are monoclonal antibodies ("mAbs"). mAbs can be produced, for example, by hybridoma technologies, recombinant technologies, phage display technologies, synthetic technologies, e.g., CDR-grafting, or combinations of such or other technologies known in the art. As referred to herein, mAbs are antibodies derived from a single copy or clone including, for example, any eukaryotic, prokaryotic or phage clone, and not the method by which it is produced.

Methods of producing and purifying antibodies are well known in the art. For example, a phage library can be screened, whereby thousands of Fab fragments are screened for interaction with recombinant human PACAP. Resulting interactions can be recovered, purified, and the amino acid sequences determined using conventional methods well known in the art, whereby initial lead antibodies can be constructed. The antibodies of the present invention are engineered to contain one or more human framework regions. Human framework germline sequences can be obtained from ImMunoGeneTics (INGT) via their website, <http://imgt.cines.fr>, or from *The Immunoglobulin FactsBook* by Marie-Paule Lefranc and Gerard Lefranc, Academic Press, 2001, ISBN 012441351. According to particular embodiments, germline HC and LC framework regions for use in the antibodies of the present invention include 3-23 and O18, respectively.

In particular embodiments of the present invention, the antibody, or the nucleic acid encoding same, is provided in isolated form. As used herein, the term "isolated" refers to a protein, peptide, or nucleic acid which is free or substantially free from other macromolecular species found in a cellular environment.

The antibodies of the present invention may be prepared and purified using known methods. For example, cDNA sequences encoding a HC (for example the amino acid

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sequence given by SEQ ID NO.1) and a LC (for example, the amino acid sequence given by SEQ ID NO.2) may be cloned and engineered into a GS (glutamine synthetase) expression vector. The engineered immunoglobulin expression vector may then be stably transfected into CHO cells. As one of skill in the art will appreciate, mammalian

5 expression of antibodies will result in glycosylation, typically at highly conserved N-glycosylation sites in the Fc region. Stable clones may be verified for expression of an antibody specifically binding to human PACAP. Positive clones may be expanded into serum-free culture medium for antibody production in bioreactors. Media, into which an antibody has been secreted, may be purified by conventional techniques. For example,

10 the medium may be conveniently applied to a Protein A or G Sepharose FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline. The column is washed to remove nonspecific binding components. The bound antibody is eluted, for example, by pH gradient and antibody fractions are detected, such as by SDS-PAGE, and then pooled. The antibody may be concentrated and/or sterile filtered using

15 common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The product may be immediately frozen, for example at -70°C, or may be lyophilized.

The antibodies of the present invention can be used in the treatment of patients.

20 More particularly the antibodies of the present invention are expected to treat a class of pain, which specifically includes headache, both primary and secondary, and migraine including chronic migraine. Although antibodies of the present invention are expected to be useful in the treatment of pain, including primary and secondary headache and migraine, such antibodies may also be useful in the treatment of other pain. As used

25 interchangeably herein, “treatment” and/or “treating” and/or “treat” are intended to refer to all processes wherein there may be a slowing, interrupting, arresting, controlling, stopping, or reversing of the progression of the disorders described herein, but does not necessarily indicate a total elimination of all disorder symptoms. Treatment includes administration of an antibody of the present invention for treatment of a disease or

30 condition in a human that would benefit from a reduction in PACAP activity, and includes: (a) inhibiting further progression of the disease, *i.e.*, arresting its development; (b) relieving the disease, *i.e.*, causing regression of the disease or disorder or alleviating

symptoms or complications thereof; and (c) preventing the onset of the disease of symptoms.

As used interchangeably herein, the term “patient,” “subject,” and “individual,” refers to a human. In certain embodiments, the patient is further characterized with a disease, disorder, or condition (e.g., pain, for example primary or secondary headache and/or migraine including chronic migraine) that would benefit from a reduction in PACAP activity. In another embodiment, the patient is further characterized as being at risk of developing a condition described above, or condition that would benefit from a reduction in PACAP activity.

As used herein, the term “bind (or binds to)” refers to an interaction of an antibody with an epitope of human PACAP. The term “epitope” as used herein refers to discrete, three-dimensional sites of an antigen that are recognized by the antibodies of the present invention.

An antibody of the present invention can be incorporated into a pharmaceutical composition which can be prepared by methods well known in the art and comprise an antibody of the present invention and one or more pharmaceutically acceptable carrier(s) and/or diluent(s) (e.g., Remington, The Science and Practice of Pharmacy, 22nd Edition, Loyd V., Ed., Pharmaceutical Press, 2012, which provides a compendium of formulation techniques as are generally known to practitioners). Suitable carriers for pharmaceutical compositions include any material which, when combined with the antibody of the present invention, retains the molecule’s activity and is non-reactive with the patient’s immune system.

A pharmaceutical composition comprising an antibody of the present invention can be administered to a patient at risk for, or exhibiting, diseases or disorders as described herein by parental routes (e.g., subcutaneous, intravenous, intraperitoneal, intramuscular, or transdermal). A pharmaceutical composition of the present invention contains an “effective” or “therapeutically effective” amount, as used interchangeably herein, of an antibody of the present invention. An effective amount refers to an amount necessary (at dosages and for periods of time and for the means of administration) to achieve the desired therapeutic result. An effective amount of the antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. An effective

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amount is also one in which any toxic or detrimental effects of the antibody of the present invention are outweighed by the therapeutically beneficial effects.

Examples

Example 1. Engineering and Expression of Exemplified Anti-PACAP Antibodies

Significant problems associated with chemical and physical stability were encountered when constructing an anti-PACAP antibody of the present invention. For example, problems encountered with initial humanized constructs include low binding affinity, unacceptable immunogenicity, variable region deamidation, oxidation, isomerization and low potency.

Chemical and physical modifications were therefore engineered to improve the binding affinity, eliminate or reduce HC dimerization, reduce immunogenicity, and improve chemical and physical stability of the antibodies of the present invention. Amino acid modifications were engineered throughout both the heavy and light chains.

Extensive protein stability studies were also performed and the constructed antibodies were screened for expression and thermostability properties as well as other properties including binding affinity. The following antibodies, which include numerous modifications from original constructs, are identified as possessing high binding affinity, being chemically and physically stable, possessing low immunogenicity and possessing pharmacokinetic properties consistent with monthly administration. None of the modifications comprising the antibodies of the present invention were identified in initially humanized constructs.

Exemplified anti-PACAP antibodies of the present invention are presented in Table 1. The exemplified antibodies include heavy chains of SEQ ID NO: 1 and light chains of SEQ ID NO: 2 as well as human HC framework 3-23 and human kappa LCs with framework O18. Additionally, engineered modifications within both the light and heavy chains which improve chemical and physical stability as well as functional properties of the antibodies are provided in Table 1. The relationship of the various regions of the exemplified anti-PACAP antibodies is as follows (numbering of amino acids applies linear numbering; assignment of amino acids to variable domains is based on the International Immunogenetics Information System[®] available at www.imgt.org; assignment of amino acids to CDR domains is based on the well-known North numbering

convention, with the exception of HCDR2 which is based on the well-known Kabat numbering convention):

Table 1: Amino acid regions of exemplified anti-PACAP antibodies of the present invention.

SEQ ID NO:1			SEQ ID NO:2		
HCVR	Region	Positions	LCVR	Region	Positions
	FRH1	1-22		FRL1	1-23
	HCDR1	23-35		LCDR1	24-34
	FRH2	36-49		FRL2	35-48
	HCDR2	50-66		LCDR2	49-56
	FRH3	67-96		FRL3	57-88
	HCDR3	97-112		LCDR3	89-97
	FRH4	113-123		FRL4	98-107
Constant	CH	124-449	Constant	CL	108-214
Exemplified Ab A	62D ; 104N ; 231P ; 237A ; 238A		Exemplified Ab A	30S ; 55L	
Exemplified Ab B	62A ; 104T ; 231P ; 237A ; 238A		Exemplified Ab B	30W ; 55F	
Exemplified Ab C	62E ; 104T ; 231P ; 237A ; 238A		Exemplified Ab C	30W ; 55F	
Exemplified Ab D	62Q ; 104T ; 231P ; 237A ; 238A		Exemplified Ab D	30W ; 55F	

The following Examples and assays demonstrate that the antibodies of the present invention are useful for treating pain including primary and secondary headache and migraine. It should be understood however, that the following Examples are set forth by way of illustration and not limitation, and that various modifications may be made by one of ordinary skill in the art.

The exemplified anti-PACAP antibodies of the present invention can be expressed and purified essentially as follows. A glutamine synthetase (GS) expression vector containing a DNA sequence encoding a HC amino acid sequence according to Table 1 (for example, a DNA sequence of SEQ ID NO:11 encoding a HC of Exemplified Antibody B presented in Table 1) and a DNA sequence encoding a LC amino acid sequence according to Table 1 (for example, a DNA sequence of SEQ ID NO:12

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encoding a LC of Exemplified Antibody B presented in Table 1) is used to transfect a Chinese hamster ovary cell line (CHO) by electroporation. The expression vector encodes an SV Early (Simian Virus 40E) promoter and the gene for GS. Expression of GS allows for the biochemical synthesis of glutamine, an amino acid required by the CHO cells. Post-transfection, cells undergo bulk selection with 50 μ M L-methionine sulfoximine (MSX). The inhibition of GS by MSX is utilized to increase the stringency of selection. Cells with integration of the expression vector cDNA into transcriptionally active regions of the host cell genome can be selected against CHO wild type cells, which express an endogenous level of GS. Transfected pools are plated at low density to allow for close-to-clonal outgrowth of stable expressing cells. The masterwells are screened for antibody expression and then scaled up in serum-free, suspension cultures to be used for production. Clarified medium, into which the antibody has been secreted, is applied to a Protein A affinity column that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column is washed with 1M NaCl to remove nonspecific binding components. Bound antibody is eluted, for example, with sodium citrate at pH (approx.) 3.5 and fractions are neutralized with 1M Tris buffer. Antibody fractions are detected, such as by SDS-PAGE or analytical size-exclusion, and then are pooled. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. Exemplified anti-PACAP antibody of the present invention is concentrated and/or sterile filtered using common techniques. The purity of exemplified antibody after these chromatography steps is greater than 95%. Exemplified anti-PACAP antibody of the present invention may be immediately frozen at -70°C or stored at 4°C for several months.

Example 2. Binding Affinity

Binding affinity (K_D) for each antibody-antigen complex is determined using a kinetic exclusion 96-well plate-based assay (adapted from Estep et al., mAbs, 2013, 5:270-278). Briefly, a 3-fold dilution series for each Exemplified Ab (set forth in Table 1) is prepared from a starting concentration of 7290pM to 41fM; each series including an antibody-blank control. Samples are prepared in 3% (w/v) Blocker A solution (MSD, #R93AA-1) and a fixed final antigen concentration of 30pM of N-terminal biotinylated

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PACAP27 (custom synthesis, CPC Scientific) or N-terminal biotinylated PACAP38 (Anaspec, #23590) is added to each sample. A volume of 100µl of each antigen-antibody sample is added to individual wells of a 96-well microtiter plate (Greiner, EK-20101) in duplicate. The plate is sealed with optical adhesive film (Thermo Fisher Scientific, #4311971) and incubated at 37°C for 3 to 4 days to allow for sample equilibrium. On the day before analysis, each row of a 96-well MSD Standard plate (MSD, #L15XA) is coated with 30µl of the corresponding antibody (as used in the titration series) at a concentration of 3µg/ml in phosphate buffered saline (PBS). On the day of the experiment, the MSD Standard plate is washed once with 150µl PBS and blocked with 150µl of 3% Blocker A solution for 45 min at 30°C with shaking at 300 rpm on a MaxQ 4450 benchtop shaker (Thermo Fisher Scientific). Following three washes with PBS, 50µl of each antigen-antibody sample (prepared and incubated as described above) is added to the MSD Standard plate and incubated for 150 seconds at 30°C with shaking at 300 rpm. Following a single wash with PBS, 50µl of 1µg/ml SULFO-TAG labeled Streptavidin (MSD, #R32AD-5) prepared in 1% (w/v) Blocker A solution is added and the MSD Standard plate and the plate is incubated at 30°C for 3 minutes with shaking at 300 rpm. The plate is washed three more times with PBS followed by the addition of 150µl/well of 1X Read buffer (MSD, #R92TC-2). The MSD Standard plate is read using a MESO Quickplex SQ 120/1300 instrument (Meso Scale Discovery). Data analysis is done using SigmaPlot (Version 12.5, Systat Software) and binding affinity (K_D) is determined using SigmaPlot's integrated Four Parameter Logistic Curve Model. Results are provided in Table 2.

Table 2: Binding affinity (K_D) of antibody-antigen complexes at 37°C

Antibody	Binding Affinity, K_D (pM, 37°C)	
	Antigen (PACAP38)	Antigen (PACAP27)
Exemplified Ab A	<i>not determined</i>	37.0
Exemplified Ab B	12.8	11.6
Exemplified Ab C	14.6	14.5
Exemplified Ab D	9.0	10.5

Example 3. In vivo PACAP Degradation

Degradation of circulating PACAP38, *in vivo*, is assessed in rats. One of control IgG4 antibody (10 mg/kg) or Exemplified Antibody A (10 mg/kg) is injected into rats intravenously. Baseline PACAP38, in plasma, is assessed for each rat and then rats are

5 injected, intravenously, with 6µg/kg PACAP38. Following PACAP38 injection, plasma samples are collected over a 120 minute period and total levels of PACAP38 (bound or unbound to antibody) are determined via a modified ELISA format. Briefly, a 96 well MSD plate (MSD, catalog #: L15xA-1) is coated with 30 µl/well of 1µg/ml anti-PACAP38 antibody (US Biological Cat# P1775-03C) in PBS and incubated overnight at

10 40°C. The plate is washed three times with 200 µl/well PBST followed by blocking with 150 µl/well 3% Blocker A (MSD Cat# R93BA-2) in PBS at room temperature for 1 hour with rotation at 650rpm. Thereafter, the plate is washed three times with 200 µl/well PBST. Calibrator of PACAP38 (Bachem Cat# H430-0500) is diluted at 1000 pg/mL with Assay Buffer containing one part diluent 2 (MSD Cat# R51-BB-3) and thirty-nine parts

15 diluent 3 (MSD Cat# R51BA-3) supplemented with 200 µg/ml HBR1 (Scantibodies Inc, Cat# 3KC533) and 10 µg/ml of Biotin-Exemplified Antibody A. Calibrator standard curve is prepared by three-fold serial dilution of 1000 pg/mL calibrator with Assay Buffer. Plasma samples are diluted 40 fold in diluent 3 supplemented with 200 µg/mL HBR1 and 10 µg/mL of Biotin-Exemplified Antibody A. 25 µl of different

20 concentrations of calibrator and diluted plasma samples are added to individual wells of the assay plate and incubated at room temperature for 2 hours with rotation at 650 rpm. After washing the plate 3 times with 200 µl/well PBST, 25 µl of 0.5 µg/ml Sulfo-tag labeled goat anti-human IgG (MSD Cat# R32AJ-1) in diluent 3 is added to each well and the plate is incubated at room temperature for 1 hour. Plates are then washed three times

25 with 200 µl/well PBST and 150 µl of 2X read buffer T (MSD, Cat# R92TC-3) is added to each well and signal is read with a MSD plate reader (results, presented as detectable PACAP38 levels, mean ± SEM (n=2-3)). Results are presented in Table 3.

Table 3: PACAP38 plasma levels

Antibody	PACAP38 Plasma Levels (ng/mL) at time points post PACAP38 injection (mean $\pm$ SEM (N))					
	baseline	5 mins.	30 mins.	60 mins.	90 mins.	120 mins.
Exemplified Ab A	0.4 $\pm$ 0.0 (2)	200.0 $\pm$ 23.0 (3)	74.1 $\pm$ 11.8 (3)	37.8 $\pm$ 3.2 (3)	20.7 $\pm$ 2.9 (3)	11.7 $\pm$ 2.5 (2)
Control IgG4 Ab	0.5 $\pm$ 0.5 (3)	6.2 $\pm$ 2.5 (3)	2.3 $\pm$ 0.9 (3)	1.1 $\pm$ 0.3 (3)	0.5 $\pm$ 0.2 (3)	0.3 $\pm$ 0.1 (3)

Results presented in Table 3 demonstrate Exemplified Antibody A prevents PACAP38 distribution upon intravenous injection but does not prevent PACAP38 *in vivo* degradation.

Example 4. Inhibition of PACAP27, PACAP38, or VIP-induced cAMP

Neutralization of PACAP27, PACAP38, and VIP induced cAMP stimulation, by the antibodies of the present invention, is assessed in CHO-K1 cells (ATCC, catalog # CCL-61) transfected with vectors expressing either human PAC 1 (NP_001186564.1) or human VPAC2 (NP_003373.2). CHO-K1 cells are harvested with nonenzymatic Cell Dissociation Buffer (Gibco #313131-014), counted, centrifuged and resuspended in assay buffer to 10,000 cells / 25 μ l. Concentrated cells (10,000 in 25 μ l) are added to wells of a 96-well plate.

Exemplified Antibodies (set forth in Table 1) are serial diluted in 100 μ l assay buffer (HBSS with calcium and magnesium (Hyclone, ThermoScientific, SH30268), 0.1 % BSA (Sigma-Aldrich, #A7888), 500 μ M IBMX (Sigma-Aldrich, I5879)), to 4-times desired final concentration. Diluted Exemplified Antibodies are incubated with agonist (at 2X the final concentration) for 15 minutes at room temperature. Final concentrations of agonist: 100 pM for PACAP27 and PACAP38 in the human PAC1, 800 pM PACAP27 and PACAP38 in the human VPAC2, 800 pM VIP in the human VPAC2 assays.

Thereafter, 25 μ l of Exemplified Antibody–agonist solution is added to individual wells of the 96-well plate (containing cells) and incubated for 1 hour at room temperature. cAMP formation in each well is determined using cAMP femto 2 (Cisbio, 62AM5PEC).

25 μ l cAMP-d2 working solution (Cisbio, 62AM5PEC) is added to each well followed by the addition of 25 μ l Anti cAMP-Cryptate working solution and the plate is incubated for 1 hr at room temperature in the dark. Resulting HTRF signal is measured at 665 and 620

nm using an Envision 12 (Perkin Elmer, excitation 330 nm) instrument, and the ratio of 665/620 is plotted to quantify cAMP (data are plotted using Graphpad Prism 7 and IC50 values are determined using the integrated Four Parameter Logistic Curve Model fitting routines). Results are provided in Table 4 as mean $\pm$ SEM.

5

Table 4: In Vitro Neutralization of PACAP27 and PACAP38 Induced cAMP Increase

Antibody	Inhibition (IC50) (pM, mean $\pm$ SEM (N))				
	PACAP38 (100 pM) in hPAC1	PACAP27 (100 pM) in hPAC1	PACAP38 (800 pM) in hVPAC2	PACAP27 (800 pM) in hVPAC2	VIP (800 pM) in hVPAC2
Exemplified Ab A	554 $\pm$ 264 (N=2)	2250 (N=1)	714 $\pm$ 194 (N=6)	781 $\pm$ 340 (N=2)	>100,000 (N=1)
Exemplified Ab B	265 $\pm$ 62 (N=3)	270 $\pm$ 103 (N=2)	294 $\pm$ 60 (N=3)	605 $\pm$ 135 (N=2)	>100,000 (N=1)
Exemplified Ab C	303 $\pm$ 15 (N=3)	311 $\pm$ 29 (N=2)	325 $\pm$ 99 (N=3)	595 $\pm$ 219 (N=2)	>100,000 (N=1)
Exemplified Ab D	205 $\pm$ 35 (N=3)	212 $\pm$ 42 (N=2)	243 $\pm$ 112 (N=3)	515 $\pm$ 127 (N=2)	>100,000 (N=1)

Example 5. Immunogenicity Analysis

10 Immunogenicity assessment, in silico, is performed with EpiMatrix T-cell epitope prediction software (within the Interactive Screening and Protein Reengineering Interface (ISPRI) web-based portal (Epivax, Inc.)) including Tregitope-adjusted Epimatrix Scores. Light chain variable region (LCVR) and heavy chain variable region (HCVR) protein sequences associated with each antibody are separately analyzed. According to Epivax,

15 protein sequences with Tregitope-adjusted Epimatrix scores greater than zero have a higher overall immunogenic potential. EpiMatrix Cluster Immunogenicity assessment is also performed for each LCVR and HCVR to identify T-cell epitope clusters within the CDR of each antibody. The presence of T-cell epitope clusters, as defined by the presence of 2 or more adjacent EpiBars, is associated with a higher overall immunogenic potential.

20 Using the North CDR definition, no CDR-related T-cell epitope clusters are identified in Exemplified Antibodies A, B, or C. A T-cell Epitope cluster of 4 adjacent EpiBars is identified in CDR2 of the LCVR of comparator antibody ALD 1.H (described as “Ab 1.H” in U.S. Patent Publication Number US 2016/0304604) indicating that the

comparator antibody has higher immunogenic potential. Results are provided in Tables 5A, 5B and 5C.

Table 5A: *In Silico* immunogenicity risk assessment via EpiMatrix T-cell Epitope

5 Prediction

	Epimatrix Score		
Antibody	HCVR	LCVR	HCVR+LCVR
Exemplified Ab A	58.84	19.44	40.61
Exemplified Ab B	65.12	26.41	47.21
Exemplified Ab C	58.74	26.41	43.79
Exemplified Ab D	65.49	26.41	47.41
ALD 1.H	70.11	35.2	53.16

Table 5B: Tregitope-Adjusted Epimatrix Assessment

	Tregitope-Adjusted Epimatrix Score		
Antibody	HCVR	LCVR	HCVR+LCVR
Exemplified Ab A	-32.82	-34.22	-33.46
Exemplified Ab B	-26.54	-27.24	-26.86
Exemplified Ab C	-32.92	-27.24	-30.29
Exemplified Ab D	-26.17	-27.24	-26.67

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ALD 1.H	-28.41	1.41	-13.93
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Table 5C: *In Silico* immunogenicity risk assessment via EpiMatrix Cluster**Immunogenicity assessment**

<u>Antibody</u>	<u>CDR-related Epibar Clusters</u>
Exemplified Ab A	0
Exemplified Ab B	0
Exemplified Ab C	0
Exemplified Ab D	0
ALD 1.H	1

5 Example 6. Neutralization of Mast Cell Degranulation-induced PACAP Release

Human mast cells are differentiated in culture from human cord blood stem cells using StemSpan Media (StemCell) and SCF/IL-6. On the day of the assay, mast cells are plated at 100,000 cells per 50 µl into wells of a 96-well tissue culture plate in Tyrode's buffer (130 mM NaCl, 5 mM KCl, 1.4 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 10 mM HEPES and 0.1% BSA, pH 7.4). Cells are treated with PACAP38 or PACAP27 in the presence or absence of an Exemplified Antibody as described below.

Single point tests are carried out by adding 25 µl of 4X- concentrated Exemplified Antibody per well (final concentration of 153 µM Exemplified Antibody). Dose curve tests are carried out by adding 25 µl of 4X-concentrated antibody per well for a final dose range of 0 to 153 µM (three-fold dilutions). 25µl of 4X-concentrated PACAP38 or PACAP27 is added to each well to a final concentration 1 µM PACAP (38 or 27). Assay medium only is used as a no treatment control and IgG4 mAb is used as a negative control. Testing is done in triplicate. The 96-well plates are placed in an incubator (37°C) for 30 minutes. Following incubation, 30 µl/well of supernatant are collected and tryptase activity is measured by commercial assay (Millipore). Percent (%) tryptase release in supernatant is calculated by the following equation: ((Experimental tryptase release – vehicle control tryptase release) / (total tryptase release – vehicle control tryptase release) x 100, where total tryptase release is obtained by freeze/thawing mast cells). Results are provided in Tables 6A, 6B and 6C.

Table 6A: Single Point Inhibition of PACAP (27 and 38) Induced Tryptase Release in Human Mast Cells

Antibody	Percentage Tryptase Release upon addition of PACAP38 or 27 (%)	
	PACAP38	PACAP27
Exemplified Ab B	4.9 ± 1.1	21 ± 1.4
Exemplified Ab C	5.5 ± 1.4	14.2 ± 1.1
Exemplified Ab D	4.2 ± 0.7	15.1 ± 4.0
Assay Medium only	-0.3 ± 0.3	-0.3 ± 0.3
IgG4 mAb	103.6 ± 0.5	109.7 ± 0.4

5 **Table 6B: Dose Curve Inhibition of PACAP27 Induced Tryptase Release in Human Mast Cells**

Antibody	Dose (uM)	Percentage Tryptase Release upon addition of PACAP27 (%)
Exemplified Ab B	153	10.3 ± 0.9
	51	19.4 ± 6.4
	17	11.6 ± 7.1
	5.6	8.1 ± 2.9
	1.8	11.7 ± 1.6
	0.6	54.0 ± 2.0
	0.2	78.1 ± 1.0
	0	85.2 ± 3.4
Exemplified Ab C	153	9.7 ± 2.3
	51	17.8 ± 2.3
	17	8.0 ± 0.6
	5.6	11.5 ± 4.9
	1.8	10.4 ± 2.6

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	0.6	55.4 ± 6.0
	0.2	85.2 ± 1.6
	0	85.8 ± 4.8
Exemplified Ab D	153	9.4 ± 0.8
	51	26.0 ± 6.3
	17	19.8 ± 3.4
	5.6	16.4 ± 10.8
	1.8	20.3 ± 8.4
	0.6	73.0 ± 2.1
	0.2	86.5 ± 1.8
	0	92.7 ± 1.5
Assay Medium only		-0.2 ± 1.0
IgG4 mAb	153	94.8 ± 4.8

Table 6C: Dose Curve Inhibition of PACAP38 Induced Tryptase Release in Human Mast Cells

Antibody	Dose (uM)	Percentage Tryptase Release upon addition of PACAP38 (%)
Exemplified Ab B	153	2.0 ± 0.3
	51	14.6 ± 2.1
	17	14.9 ± 2.6
	5.6	22.0 ± 4.1
	1.8	25.2 ± 1.4
	0.6	52.4 ± 9.3
	0.2	73.7 ± 1.3
	0	77.2 ± 1.0
Exemplified Ab C	153	2.8 ± 0.3
	51	12.1 ± 2.3
	17	15.5 ± 0.9
	5.6	21.9 ± 3.8

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	1.8	26.5 ± 4.3
	0.6	66.6 ± 1.0
	0.2	77.6 ± 0.3
	0	80.3 ± 2.6
Exemplified Ab D	153	1.7 ± 0.1
	51	14.7 ± 1.5
	17	19.6 ± 3.6
	5.6	25.9 ± 6.2
	1.8	31.3 ± 3.8
	0.6	64.5 ± 4.1
	0.2	74.0 ± 1.8
	0	76.5 ± 0.7
Assay Medium only		-0.2 ± 1.0
IgG4 mAb	153	79.6 ± 4.1

Example 7. *In Vivo* PACAP-induced cAMP Neutralization

Neutralization of PACAP-induced plasma cAMP increase, *in vivo*, is assessed in CD-1 murine model (Envigo). Briefly, male CD-1 mice are subcutaneously administered 10 mg/kg of one of: Control IgG4 antibody (N= 6); Control IgG4 antibody plus PBS/rolipram only (N= 5); Exemplified Antibody B (N=5); Exemplified Antibody C (N=5); or Exemplified Antibody D (N=6). Three days-post antibody treatment, mice are intravenously administered PACAP38 (13 nmols/kg) plus rolipram (100ug/mL); some mice previously treated with IgG4 control antibody are intravenously administer PBS (containing 0.2% ethanol, 1% BSA, 5 ml/kg, and rolipram (100 µg/ml) only. Ten minutes post-PACAP38 treatment, blood is collected in EDTA containing tubes and plasma is separated by centrifugation. Plasma levels of cAMP are determined using commercially available cAMP ELISA (Cell Bios, Inc.) according to manufacturer instructions. Statistical analysis applies log transformed cAMP concentration in a one-way ANOVA (Graphpad Prism 7) followed by Dunnett's post hoc analysis. Results are set forth in Table 7 as mean ± SE.

Table 7: PACAP-Induced Plasma cAMP Levels

Antibody	Plasma cAMP levels (nM, mean $\pm$ SEM)
IgG4 Control Ab (plus PBS/rolipram only)	238 $\pm$ 60 (N=5) *
IgG4 Control Ab (plus PACAP)	4095 $\pm$ 318 (N=6)
Exemplified Ab B (plus PACAP)	715 $\pm$ 60 (N=5) *
Exemplified Ab C (plus PACAP)	666 $\pm$ 96 (N=5) *
Exemplified Ab D (plus PACAP)	560 $\pm$ 130 (N=6) *

*p<0.0001 vs. IgG4 Control Ab (PACAP38)

Example 8. Neutralization of Trigeminal Ganglia Stimulation-Induced Dural

5 Plasma Protein Extravasation *In Vivo*

The ability of anti-PACAP antibodies of the present invention to block, *in vivo*, plasma protein extravasation induced by release of PACAP following stimulation of the trigeminal ganglia is examined using a rat model. Briefly, Sprague-Dawley rats (Envigo, males, 250-350 g) are subcutaneously administered one of: 1-10 mg/kg of Exemplified

10 Antibody A; 3-30 mg/kg of Exemplified Antibody B; or 3 or 10 mg/kg of Control IgG4 Ab. Seventy-two hours later, rats are anesthetized with Nembutal (65 mg/kg, ip.) and placed in a stereotaxic frame (David Kopf Instruments) with the incisor bar set at -2.5 mm. A mid-line sagittal scalp incision is made, followed by two pairs of bilateral holes drilled through the skull (3.2 mm posteriorly, 1.8 and 3.8 mm laterally, all coordinates

15 referenced to bregma). Pairs of stainless steel stimulating electrodes (Rhodes Medical Systems, Inc.) are lowered through the holes in both hemispheres to a depth of 9.2 mm. from the dura. Two-minutes prior to trigeminal ganglion stimulation, fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) (20 mg/kg, iv.), a marker for protein extravasation, is injected into the femoral vein. The left trigeminal ganglion is

20 stimulated for 5 minutes at a current intensity of 1.0 mA (5 Hz, 5 msec pulse duration) with a Model S48 Grass Instrument Stimulator with PSIU6 photoelectric isolation unit (Grass-Telefactor). Five minutes following stimulation, the rats are sacrificed by exsanguination with 40 ml of saline.

Following sacrifice of the rats, the top of the skull is removed and dural membrane is collected. Membrane samples are removed from both hemispheres, rinsed with water, and spread flat on microscope slides. Slides are dried for 15 minutes on a slide warmer and tissues are cover-slipped with a 70% glycerol/water solution. A fluorescence microscope (Zeiss) equipped with a grating monochromator and a spectrophotometer are used to quantify FITC-BSA dye in each dural sample. The extravasation induced by electrical stimulation of the trigeminal ganglion is an ipsilateral effect (i.e. occurs only on the side of the dura in which the trigeminal ganglion is stimulated), thus allowing the unstimulated half of the dura to serve as a control. The ratio of extravasation in the dura from the stimulated side versus the unstimulated side is calculated. Results are provided in Table 8 (mean $\pm$ SE).

Table 8: Trigeminal Ganglia Stimulation-Induced Dural Plasma Protein Extravasation

<u>Antibody</u> (mg/kg)	<u>Extravasation Ratio</u> (mean $\pm$ SEM)
IgG4 Control Ab (10 mg/kg) (N=3)	1.87 $\pm$ 0.04
Exemplified Ab A (1 mg/kg) (N=3)	1.93 $\pm$ 0.04
Exemplified Ab A (3 mg/kg) (N=3)	1.59 $\pm$ 0.07
Exemplified Ab A (10 mg/kg) (N=3)	1.13 $\pm$ 0.05
IgG4 Control Ab (3 mg/kg) (N=3)	1.82 $\pm$ 0.02
IgG4 Control Ab (10 mg/kg) (N=3)	1.90 $\pm$ 0.04
IgG4 Control Ab (30 mg/kg) (N=3)	1.95 $\pm$ 0.05
Exemplified Ab B (3 mg/kg) (N=3)	1.83 $\pm$ 0.06
Exemplified Ab B (10 mg/kg) (N=3)	1.21 $\pm$ 0.06
Exemplified Ab B (30 mg/kg) (N=3)	1.07 $\pm$ 0.04

Example 9. Pharmacokinetics of Exemplified Anti-PACAP Antibodies

Serum pharmacokinetics of exemplified antibodies of the present invention are characterized in male cynomolgus monkeys (n=2) following a single SC administration by total IgG assay. Animals are injected subcutaneous with Exemplified Antibody B

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(10mg/kg) and serum samples collected at 1, 3, 6, 24, 48, 72, 96, 120, 144, 168, 240, 336, 504 and 672 hours post injection. Total IgG assay is performed generally as described herein. Briefly, 100 uL/well of 1 ug/mL goat anti-human IgG F(ab')₂ antibody (Jackson ImmunoResearch Laboratories, Inc., Catalog Number 109-006-097) is coated on an

5 Immulon 4HBX plate. Wells are incubated with one of standards (a standard curve for Exemplified Antibody B is prepared from 15.63-1,000 ng/mL), control or serum samples, followed by 100 ul/well of 1:10,000 diluted mouse anti-human IgG Fc-horseradish peroxidase (HRP; SouthernBiotech, Catalog Number 9040-05) for detection. Unbound

10 detection reagent is washed away. Thereafter, 100 ul/well TMB Microwell Peroxidase Substrate System is added to the wells. Color development is stopped by addition of 100 ul/well TMB Stop Solution and optical density is measured at 450 nm with wavelength correction set to 630 nm. Immunoreactivity is determined from known amounts of Exemplified Antibody B in 100% cynomolgus monkey serum, followed by a minimum

15 required dilution of 1:10 in Blocker™ Casein in PBS using a 5-parameter algorithm (StatLia; version 3.2). Following procedures essentially as described above, apparent clearance (CL/F) of 0.49 mL/hr/kg, apparent volume of distribution (V/F) of 114.0 mL/kg, and terminal half-life of 171 hours calculated by non-compartmental analysis are calculated for Exemplified Antibody B. The pharmacokinetic results obtained are consistent with therapeutic antibodies capable of extended duration of action.

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Sequences**Exemplified HC (SEQ ID NO.1)**

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYYMSWVRQAPGKGLEWVSAISLS
 GGSTYYAXSHKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCVREVGASXHN
 5 YGMDVWGQGTMTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVT
 VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGKTYTCNVDHKPSNTK
 VDKRVESKYGPXCPAPEXXGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSQ
 EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK
 CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS QEEMTKNQVSLTCLVKGFYPSD
 10 IAVEWESNGQPENNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHE
 ALHNHYTQKSLSLGLG

wherein, X at residue 62 is one of D, A, E and Q; X at residue 104 is one of N and T; X at residue 231 is one of P and S; X at residue 237 is one of A and F; and X at residue 238 is one of A and L.

15

Exemplified LC (SEQ ID NO.2)

DIQMTQSPSSLSASVGDRVTITCRASQSIXRWLAWYQQKPGKAPKLLIHDASQLX
 EGVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQFDLLPLTFGGGTKVEIKRTVAA
 PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
 20 SKDSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

wherein X at residue 30 is one of S and W; and X at residue 55 is one of L and F.

Exemplified HCDR1 (SEQ ID NO.3)

AASGFTFSSYYMS

25

Exemplified HCDR2 (SEQ ID NO.4)

AISLSGGSTYYAXSHKG

wherein X at residue 13 is one of D, A, E and Q.

Exemplified HCDR3 (SEQ ID NO.5)

VREVGASXHNYYGMDV

wherein X at residue 8 is one of N and T.

Exemplified LCDR1 (SEQ ID NO.6)

35 RASQSIXRWLA

wherein X at residue 7 is one of S and W.

Exemplified LCDR2 (SEQ ID NO.7)

HDASQLXE

wherein X at residue 7 is one of L and F.

5

Exemplified LCDR3 (SEQ ID NO.8)

QQFDLLPLT

Exemplified HCVR (SEQ ID NO.9)

10 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYYMSWVRQAPGKGLEWVSAISLS
GGSTYYAXSHKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCVREVGASXHN
YGM DVWGQGTMTVSS

wherein, X at residue 62 is one of D, A, E and Q; and X at residue 104 is one of N and T.

Exemplified LCVR (SEQ ID NO.10)

15 DIQMTQSPSSLSASVGDRVTITCRASQSIXRWLAWYQQKPGKAPKLLIHDASQLX
EGVPSRFSGSGSGTDFTFTISSLQPEDATYYCQQFDLLPLTFGGGGTKVEIK

wherein X at residue 30 is one of S and W; and X at residue 55 is one of L and F.

20 Nucleotide Sequence Encoding HC of Exemplified Ab B (SEQ ID NO.11)

gaggtgcagctgttgagctgggggagggc ttgtacagcctggggggtccctgagactc
tctgtgcagcctctggattcaccttagc agctattacatgagctgggtccgccaggct
ccagggaaggggctggagtgggtctcagct attagtctgagtggtgtagcacatactac
gcagcgtcccacaagggccggtcaccatc tccagagacaattccaagaacacgctgtat
25 ctgcaaatgaacagcctgagagccgaggac acggccgtatattactgtgtccgggaggtg
ggagctagcactcacaactactacggtatg gacgtctggggccaagggaacctggtcacc
gtctcttcagcttctaccaagggcccatcg gtcttcccgtagcgcctgtccaggagc
acctccgagagcacagccgcccctgggctgc ctggtcaaggactacttccccgaaccggtg
acgggtgtcgtggaactcaggcgcctgacc agcggcgtgcacaccttccggtgtccta
30 cagtctcaggactctactccctcagcagc gtggtgaccgtgccctccagcagcttgggc
acgaagacctacacctgcaacgtagatcac aagcccagcaacaccaaggtggacaagaga
gttgagtccaaatatgggtcccatgccc cctgcccagcacctgaggccgcccggggga
ccatcagttctgttcccccaaaaccc aaggacactctcatgatctccggaccct
gaggtcacgtgcgtgggtggacgtgagc caggaagaccccaggtccagttcaactgg
35 tacgtggatggcgtggaggtgcataatgcc aagacaaagccgaggaggagcagttcaac
agcacgtaccgtgtggtcagcgtcctcacc gtctgcaccaggactgggtgaacggcaag
gagtacaagtgcgaaggttccaacaaaggc ctcccgtctccatcgagaaaaccatctcc
aaagccaaagggcagccccgagagccacag gtgtacacctgccccatcccaggaggag
atgaccaagaaccaggtcagcctgacctgc ctggtcaaaggcttctacccagcgacatc
40 gccgtggagtggaagcaatgggcagccg gagaacaactacaagaccacgcctccctg

-27-

ctggactccgacggctccttctctctac agcaggctaaccgtggacaagagcaggtgg
 caggaggggaatgtcttctcatgtccgtg atgcatgaggctctgcacaaccactacaca
 cagaagagcctctccctgtctctgggt

5 **Nucleotide Sequence Encoding LC of Exemplified Ab B (SEQ ID NO.12)**

gacatccagatgaccagtcctccatctcc ctgtctgcatctgtaggagacagagtcacc
 atcacttgccggcgagtcagagtatttgg aggtggttggcctggatcagcagaaacca
 gggaaagcccctaagctcctgatccacgat gcatcccaattgtcgaaggggtcccatca
 aggttcagtggagtgatctgggacagat ttactttcaccatcagcagcctgcagcct
 10 gaagatattgcaacatattactgtcaacag ttgatttgctccctctcactttcggcgga
 gggaccaaggtggagatcaaacggaccgtg gctgcaccatctgtcttcatttcccgcca
 tctgatgagcagttgaaatctggaactgcc tctgtgtgtgcctgctgaataacttctat
 cccagagaggccaaagtacagtgggaaggtg gataacgccctccaatcgggtaactcccag
 gagagtgtcacagagcaggacagcaaggac agcacctacagcctcagcagcaccctgacg
 15 ctgagcaaagcagactacgagaaacacaaa gtctacgcctgcgaagtcacccatcagggc
 ctgagctcgcccgtcacaagagcttcaac
 aggggagagtgc

Amino Acid Sequence of Recombinant Human PACAP 38 (SEQ ID NO.13)

20 HSDGIFTDSYSRYRKQMAVKKYLA AVLGLKRYKQRVKNK

wherein K at residue 38 is post-translationally modified by C-terminal amidation

Amino Acid Sequence of Recombinant Human PACAP 27 (SEQ ID NO.14)

HSDGIFTDSYSRYRKQMAVKKYLA AVL

25 wherein L at residue 27 is post-translationally modified by C-terminal amidation

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WE CLAIM:

1. An antibody that binds human pituitary adenylate cyclase-activating peptide comprising a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the HCVR comprises complementarity determining regions (CDRs)
5 HCDR1, HCDR2, and HCDR3 and the LCVR comprises CDRs LCDR1, LCDR2, and LCDR3, wherein the amino acid sequence of HCDR1 is SEQ ID NO.3, the amino acid sequence of HCDR2 is SEQ ID NO.4, the amino acid sequence of HCDR3 is SEQ ID NO.5, the amino acid sequence of LCDR1 is SEQ ID NO.6, the amino acid sequence of LCDR2 is SEQ ID NO.7, and the amino acid sequence of LCDR3 is SEQ
10 ID NO.8.
2. The antibody of Claim 1, wherein HCDR2 comprises aspartic acid at residue 13; HCDR3 comprises asparagine at residue 8; LCDR1 comprises serine at residue 7; and LCDR2 comprises leucine at residue 7.
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3. The antibody of Claim 1, wherein HCDR2 comprises alanine at residue 13; HCDR3 comprises threonine at residue 8; LCDR1 comprises tryptophan at residue 7; and LCDR2 comprises phenylalanine at residue 7.
- 20 4. The antibody of Claim 1, wherein HCDR2 comprises glutamic acid at residue 13; HCDR3 comprises threonine at residue 8; LCDR1 comprises tryptophan at residue 7; and LCDR2 comprises phenylalanine at residue 7.
5. The antibody of Claim 1, wherein HCDR2 comprises glutamine at residue 13;
25 HCDR3 comprises threonine at residue 8; LCDR1 comprises tryptophan at residue 7; and LCDR2 comprises phenylalanine at residue 7.
6. The antibody of Claim 1, comprising a heavy chain variable region (HCVR) and a light chain variable region (LCVR), wherein the amino acid sequence of the HCVR is
30 SEQ ID NO.9 and the amino acid sequence of the LCVR is SEQ ID NO. 10.

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7. The antibody of Claim 6, wherein HCVR comprises aspartic acid at residue 62 and asparagine at residue 104, and wherein LCVR comprises serine at residue 30 and leucine at residue 55.
- 5 8. The antibody of Claim 6, wherein HCVR comprises alanine at residue 62 and threonine at residue 104, and wherein LCVR comprises tryptophan at residue 30 and phenylalanine at residue 55.
9. The antibody of Claim 6, wherein HCVR comprises glutamic acid at residue 62 and
10 threonine at residue 104, and wherein LCVR comprises tryptophan at residue 30 and phenylalanine at residue 55.
- 10 The antibody of Claim 6, wherein HCVR comprises glutamine at residue 62 and
threonine at residue 104, and wherein LCVR comprises tryptophan at residue 30 and
15 phenylalanine at residue 55.
11. An antibody that binds human pituitary adenylate cyclase-activating peptide comprising a heavy chain (HC) and a light chain (LC), wherein the amino acid sequence of the HC is SEQ ID NO.1 and the amino acid sequence of the LC is SEQ
20 ID NO.2.
12. The antibody of Claim 11, wherein HC comprises aspartic acid at residue 62, asparagine at residue 104, proline at residue 231, alanine at residue 237 and alanine at residue 238, and wherein LC comprises serine at residue 30 and leucine at residue 55.
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13. The antibody of Claim 11, wherein HC comprises alanine at residue 62, threonine at residue 104, proline at residue 231, alanine at residue 237 and alanine at residue 238, and wherein LC comprises tryptophan at residue 30 and phenylalanine at residue 55.
- 30 14. The antibody of Claim 11, wherein HC comprises glutamic acid at residue 62, threonine at residue 104, proline at residue 231, alanine at residue 237 and alanine at

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residue 238, and wherein LC comprises tryptophan at residue 30 and phenylalanine at residue 55.

15. The antibody of Claim 11, wherein HC comprises glutamine at residue 62, threonine at residue 104, proline at residue 231, alanine at residue 237 and alanine at residue 238, and wherein LC comprises tryptophan at residue 30 and phenylalanine at residue 55.

16. A pharmaceutical composition comprising an antibody of any one of Claims 1-15 and one or more pharmaceutically acceptable carriers, diluents or excipients.

17. A method of treating one of primary headache, secondary headache and migraine comprising administering to a patient in need thereof an effective amount of an antibody of any one of Claims 1-15.

18. The method of Claim 17, wherein the primary headache is a trigeminal autonomic cephalalgias.

19. The method of Claim 18, wherein the trigeminal autonomic cephalalgias is one of episodic cluster headache, chronic cluster headache, paroxysmal hemicranias, and unilateral neuralgiform headache attack.

20. A method of treating one of primary headache, secondary headache and migraine comprising administering to a patient in need thereof the pharmaceutical composition of Claim 16.

21. The method of Claim 20, wherein the primary headache is a trigeminal autonomic cephalalgias.

22. The method of Claim 21, wherein the trigeminal autonomic cephalalgias is one of episodic cluster headache, chronic cluster headache, paroxysmal hemicranias, and unilateral neuralgiform headache attack.

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23. An antibody of any one of Claims 1-15 for use in therapy.

24. An antibody of any one of Claims 1-15 for use in the treatment of one of primary
5 headache, secondary headache and migraine.

25. An antibody of any one of Claims 1-15 for use in the treatment of a trigeminal
autonomic cephalalgias.

10 26. The antibody of Claim 25, wherein the trigeminal autonomic cephalalgias is one of
episodic cluster headache, chronic cluster headache, paroxysmal hemicranias and
unilateral neuralgiform headache attack.

15 27. An antibody of any one of Claims 1-15 for use in the manufacture of a medicament
for the treatment of one of primary headache, secondary headache and migraine.

28. An antibody of any one of Claims 1-15 for use in the manufacture of a medicament
for the treatment of a trigeminal autonomic cephalalgias.

20 29. The antibody of Claim 28, wherein the trigeminal autonomic cephalalgias is one of
episodic cluster headache, chronic cluster headache, paroxysmal hemicranias and
unilateral neuralgiform headache attack.

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