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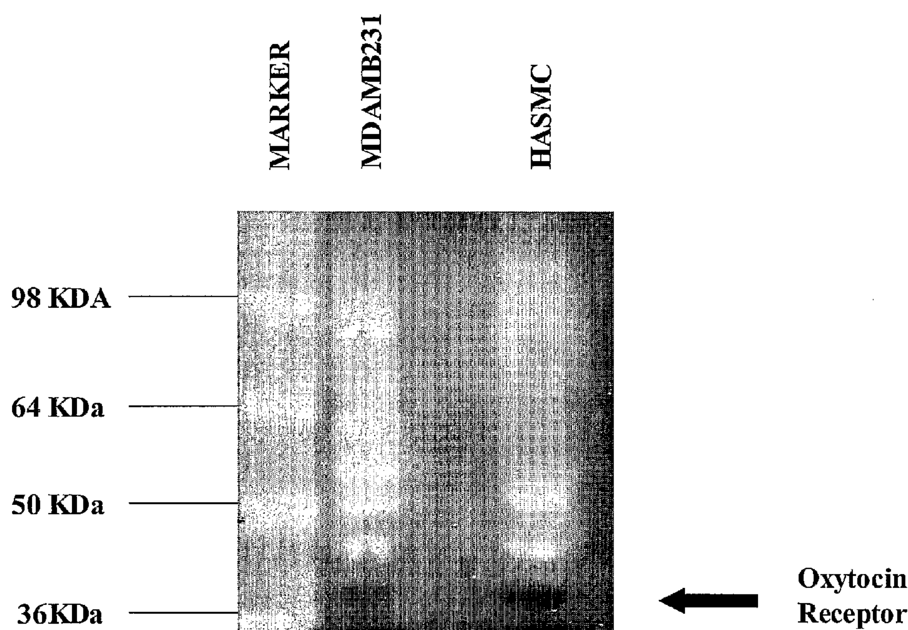
(54) Title: OXYTOCIN RECEPTOR ANTAGONISTS AND THEIR USE FOR THE TREATMENT OF PULMONARY RELATED DISEASES



(57) Abstract: Methods of treating disorders related to Oxytocin Receptor activity utilize OXYTOCIN RECEPTOR antagonists, such as antibodies, including specified portions or variants, polypeptides, polynucleotides, small molecule drugs, siRNA, shRNA, and DNAzymes. Disorders related to Oxytocin Receptor activity include inflammatory disorders, such as pulmonary disorders, for example, asthma, emphysema, and COPD.

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OXYTOCIN RECEPTOR ANTAGONISTS AND THEIR USE FOR THE TREATMENT OF PULMONARY RELATED DISEASES

FIELD OF THE INVENTION

The present invention relates to OXYTOCIN RECEPTOR antagonists and a method of using OXYTOCIN RECEPTOR antagonists to treat pulmonary disorders and symptoms, and conditions, as well as related diseases and conditions. The invention more specifically relates to methods of treating such diseases by the use of OXYTOCIN RECEPTOR antagonists, such as interfering RNA, DNAzymes, and antibodies directed toward the Oxytocin Receptor, including specified portions or variants, specific for at least one protein or fragment thereof, in an amount effective to inhibit Oxytocin Receptor activity.

BACKGROUND OF THE INVENTION

Asthma is a complex, chronic disorder, with a genetic and an environmental component (1). It is characterized by reversible airway obstruction, airway hyperresponsiveness, airway inflammation and remodeling (2). Asthma affects an estimated 15 million Americans and the morbidity and mortality associated with it is on the rise in industrialized countries (3,4). Inflammation in the airway of an allergic asthmatic is associated with the mucosal infiltration of T helper (Th)2 subset of $CD4^+$ T cells and eosinophils (5,6). The interaction between these cells leads to the production of various pro-inflammatory mediators involved in the pathogenesis of asthma (7,8). Other forms of asthma are those that are induced by exercise, viruses, aspirin and occupation. Although the mechanism responsible for these forms of asthma might involve Th2 lymphocytes and cytokines it might be triggered differently (9-12). Many cytokines and chemokines are involved in the pathogenesis of asthma (13,14). Specifically, the Th2 derived cytokines (interleukin 4, 5, 9 and 13) play an important role in allergic diseases including asthma. In an effort to identify novel genes involved in the pathogenesis of asthma researchers have used DNA microarray technology to profile genes that are differentially expressed in animal models of asthma (15,16).

Microarray technology is a powerful tool since it enables analysis of the expression of thousands of genes simultaneously and can also be automated allowing for a high-throughput format. In multifactorial diseases, such as asthma, microarray results can provide a gene expression profile which can prove very useful in designing new therapeutics. Also, it can prove very powerful in identifying novel genes and annotating genes of unknown function (17).

Oxytocin (OT), a hypothalamic neuropeptide has classically been known to induce uterine contractions during parturition and milk ejection during lactation. It mediates these functions via the Oxytocin Receptor. Just before the onset of labor, uterine muscle becomes exceedingly sensitive to oxytocin, because of a dramatic increase in the amount of Oxytocin Receptor.

Recently, this concept of the action of oxytocin has expanded due to the discovery of novel sites of expression of Oxytocin Receptor gene (OXTR), such as the pituitary, kidney, ovary, testis, thymus, heart, vascular endothelium, osteoclasts, myoblasts, pancreatic islets, adipocytes, several types of cancer cells (18), and smooth muscle and epithelial compartment of the human epididymis, where it may play a role in the ejaculatory process in males (19).

The Oxytocin receptor gene (Gene symbol OXTR, GenBank accession NM_000916, SEQ ID NO:1) resides on human chromosome 3p25. It has 4 exons and 3 introns. Exons 1 and 2 correspond to the 5-prime non-coding region, followed by exons 3 and 4 encoding the amino acids of the protein (20). The Oxytocin Receptor (GenBank accession NP_000907, SEQ ID NO: 2) belongs to the G-protein coupled receptor family. Its activity is mediated by G proteins, which activate a phosphatidylinositol-calcium second messenger system.

Gene expression can be modulated in several different ways, including by the use of siRNAs, shRNAs, antisense molecules and DNAzymes. SiRNAs and shRNAs both work via the RNAi pathway and have been successfully used to suppress the expression of genes. RNAi was first discovered in worms and the phenomenon of gene silencing related to dsRNA was first reported in plants by Fire and Mello and is thought to be a way for plant cells to combat infection with RNA viruses. In this pathway, the long dsRNA viral product is processed into smaller fragments of 21-25 bp in length by a DICER-like enzyme and then the double-stranded molecule is unwound and loaded into the RNA induced silencing complex (RISC). A similar pathway has been identified in mammalian cells with the notable difference that the dsRNA molecules must be smaller than 30 bp in length in order to avoid the induction of the so-called interferon response, which is not gene specific and leads to the global shut down of protein synthesis in the cell.

Synthetic siRNAs can be designed to specifically target one gene and they can easily be delivered to cells in vitro or in vivo. ShRNAs are the DNA equivalents of siRNA molecules and have the advantage of being incorporated into the cells' genome and then being replicated during every mitotic cycle.

DNAzymes have also been used to modulate gene expression. DNAzymes are catalytic DNA molecules that cleave single-stranded RNA. They are highly selective for the target RNA sequence and as such can be used to down-regulate specific genes through targeting of the messenger RNA.

5 Accordingly, there is a need to identify and characterize new methods for diagnosing and treatment related to the OXTR for pulmonary disorders, such as asthma, and related diseases and conditions.

Summary of the Invention

The present invention relates to agonists and/or antagonists of oxytocin, the 10 oxytocin receptor, and/or one or both of their activities (hereinafter "OXYTOCIN RECEPTOR antagonists") and a method of using OXYTOCIN RECEPTOR antagonists, including antibodies directed toward oxytocin or the Oxytocin Receptor, and specified portions or variants thereof specific for at least one Oxytocin Receptor protein or fragment thereof, to treat pulmonary-related disorders.

15 In one embodiment, the OXYTOCIN RECEPTOR antagonist is an antibody that specifically binds oxytocin or the Oxytocin Receptor. A particular advantage of such antibodies is that they are capable of binding oxytocin or the Oxytocin Receptor in a manner that prevents its action. The method of the present invention thus employs antibodies having the desirable neutralizing property which makes them ideally suited for therapeutic and preventative 20 treatment of disease states associated with various pulmonary-related disorders in human or nonhuman patients. Accordingly, the present invention is directed to a method of treating a pulmonary-related disease or condition in a patient in need of such treatment which comprises administering to the patient an amount of a neutralizing OXYTOCIN RECEPTOR antibody to inhibit the pulmonary-related disease or condition.

25 In another aspect, the invention provides methods for modulating activity of an oxytocin or its receptor polypeptide comprising contacting a cell with an agent (e.g., antagonist or agonist) that modulates (inhibits or enhances) the activity or expression of oxytocin or the Oxytocin Receptor such that activity or expression in the cell is modulated. In a preferred embodiment, the agent is an antibody that specifically binds to oxytocin or the Oxytocin 30 Receptor. In other embodiments, the modulator is a peptide, peptidomimetic, or other small molecule.

The present invention also provides methods of treating a subject having a pulmonary or related disorder wherein the disorder can be ameliorated by modulating the

amount or activity of oxytocin or the Oxytocin Receptor. The present invention also provides methods of treating a subject having a disorder characterized by aberrant activity of oxytocin or the Oxytocin Receptor or one or both of their encoding polynucleotide by administering to the subject an agent that is a modulator of the activity of oxytocin or the Oxytocin Receptor or a
5 modulator of the expression of oxytocin or the Oxytocin Receptor.

In one embodiment, the modulator is a polypeptide or small molecule compound. In another embodiment, the modulator is a polynucleotide. In a particular embodiment, the OXYTOCIN RECEPTOR antagonist is an siRNA molecule, an shRNA molecule, or a DNAzyme capable of preventing the production of Oxytocin Receptor by cells.

10 The present invention further provides any invention described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the RNA expression levels of the Oxytocin Receptor in asthmatic cells compared to non-asthmatic cells in response to treatment with atopic serum.

Fig. 2 shows the RNA expression levels of the Oxytocin Receptor in asthmatic
15 cells compared to non-asthmatic cells in response to treatment with non-atopic serum treatment.

Fig. 3 shows the expression of Oxytocin Receptor in human airway smooth muscle cells (HASMC).

Fig. 4 shows the amount of calcium released in human airway smooth muscle
20 cells in response to oxytocin either unstimulated or stimulated with IL-13 (50 ng/ml) for 24 hours.

Detailed Description of the Invention

Definitions

The following definitions are set forth to illustrate and define the meaning and
25 scope of various terms used to describe the invention herein.

An "activity," a biological activity, and a functional activity of a polypeptide refers to an activity exerted by oxytocin or the Oxytocin Receptor in response to its specific interaction with another protein or molecule as determined in vivo, in situ, or in vitro, according to standard techniques. Such activities can be a direct activity, such as an association with or an enzymatic
30 activity on a second protein, or an indirect activity, such as a cellular process mediated by

interaction of the protein with a second protein or a series of interactions as in intracellular signaling or the coagulation cascade.

An "antibody" includes any polypeptide or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to, at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion, fragment or variant thereof. The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. For example, antibody fragments include, but are not limited to, Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and partial reduction) and F(ab')2 (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Polypeptide Science, John Wiley & Sons, NY (1997-2001)).

"Chimeric" or "fusion" molecules are nucleic acids or polypeptides that are created by combining one or more OXYTOCIN RECEPTOR antagonists (or their parts) with additional nucleic acid sequence(s). Such combined sequences may be introduced into an appropriate vector and expressed to give rise to a chimeric or fusion polypeptide.

"Complement of" or "complementary to" a nucleic acid sequence of the invention refers to a polynucleotide molecule having a complementary base sequence and reverse orientation as compared to a first polynucleotide.

"Fragment" is a variant polypeptide having an amino acid sequence that is entirely the same as part but not all of any amino acid sequence of an OXYTOCIN RECEPTOR antagonist or a variant polynucleotide having a nucleic acid sequence that is entirely the same as part but not all of any nucleic acid sequence of any OXYTOCIN RECEPTOR antagonist polynucleotide. Fragments can include, e.g., truncation polypeptides, or variants thereof, such as a continuous series of residues that includes a heterologous amino- and/or carboxy-terminal amino acid sequence. Degradation forms of the OXYTOCIN RECEPTOR antagonists produced by or in a host cell are also included. Other exemplary fragments are characterized by structural or functional attributes, such as fragments that comprise alpha-helix or alpha-helix

forming regions, beta-sheet or beta-sheet forming regions, turn or turn-forming regions, coil or coil-forming regions, hydrophilic regions, hydrophobic regions, alpha-amphipathic regions, beta-amphipathic regions, flexible regions, surface-forming regions, substrate binding regions, extracellular regions, and high antigenic index regions.

5 "Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including, 10 but not limited to, those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing:Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, 15 Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., Siam J. Applied Math., 48:1073 (1988). In addition, values for percentage identity can be obtained from amino acid and nucleotide sequence alignments generated using the default settings for the AlignX component of Vector NTI Suite 8.0 (Informax, Frederick, MD).

Preferred methods to determine identity are designed to give the largest match 20 between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S. F. et al., J. Molec. Biol. 215:403-410 (1990)). The BLAST X program is 25 publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBINLM NIH Bethesda, Md. 20894: Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990). The well-known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

30 (1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48:443-453 (1970) Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci, USA. 89:10915-10919 (1992)
Gap Penalty: 12
Gap Length Penalty: 4
A program useful with these parameters is publicly available as the "gap" program from

Genetics Computer Group, Madison Wis. The aforementioned parameters are the default parameters for peptide sequence comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:
(1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48:443-453 (1970)
5 Comparison matrix: matches=+10, mismatch=0
Gap Penalty: 50
Gap Length Penalty: 3
Available as: The "gap" program from Genetics Computer Group, Madison Wis. These are the default parameters for nucleic acid sequence comparisons.

10 By way of example, a polynucleotide sequence may be identical to a sequence, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein the alterations may occur at the 5' or 3' terminal positions
15 of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in the sequence by the numerical percent of the respective percent identity (divided by 100) and subtracting that product from the
20 total number of nucleotides in the sequence, or:
n.sub.n.ltorsim.x.sub.n -(x.sub.n.y),
wherein n.sub.n is the number of nucleotide alterations, x.sub.n is the total number of nucleotides in the sequence, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, etc., and wherein any non-integer product of x.sub.n and y is
25 rounded down to the nearest integer prior to subtracting from x.sub.n.

Alterations of a polynucleotide sequence encoding the sequence may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations. Similarly, a polypeptide sequence may be identical to the reference sequence, that is be 100% identical, or it may
30 include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the percentage identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein the alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or

anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by multiplying the total number of amino acids in the sequence by the numerical percent of the 5 respective percent identity (divided by 100) and then subtracting that product from the total number of amino acids in the sequence, or:

n.sub.a.ltorsim.x.sub.a -(x.sub.a.y),

wherein n.sub.a is the number of amino acid alterations, x.sub.a is the total number of amino acids in the sequence, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and 10 wherein any non-integer produce of x.sub.a and y is rounded down to the nearest integer prior to subtracting it from x.sub.a.

"Nucleic acids" are polymers of nucleotides, wherein a nucleotide comprises a base linked to a sugar which sugars are in turn linked one to another by an interceding at least bivalent molecule, such as phosphoric acid. In naturally occurring nucleic acids, the sugar is 15 either 2'-deoxyribose (DNA) or ribose (RNA). Unnatural poly- or oliogonucleotides contain modified bases, sugars, or linking molecules, but are generally understood to mimic the complementary nature of the naturally occurring nucleic acids after which they are designed. An example of an unnatural oligonucleotide is an antisense molecule composition that has a phosphorothiorate backbone. An "oligonucleotide" generally refers to a nucleic acid molecule 20 having less than 30 nucleotides.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, and a peptide generally refers to amino acid polymers of 12 or less residues. Peptide bonds can be produced naturally as directed by the nucleic acid template or synthetically by methods well known in the art.

25 A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may further comprise substituent groups attached to the side groups of the amino acids not involved in formation of the peptide bonds. Typically, proteins formed by eukaryotic cell expression also contain carbohydrates. Proteins are defined herein in terms of their amino acid sequence or backbone and substituents are not specified, whether known or not.

30 The term "receptor" denotes a molecule having the ability to affect biological activity, in e.g., a cell, as a result of interaction with a specific ligand or binding partner. Cell membrane bound receptors are characterized by an extracellular ligand-binding domain, one or more membrane spanning or transmembrane domains, and an intracellular effector domain that is typically involved in signal transduction. Ligand binding to cell membrane receptors causes

changes in the extracellular domain that are communicated across the cell membrane, direct or indirect interaction with one or more intracellular proteins, and alters cellular properties, such as enzyme activity, cell shape, or gene expression profile. Receptors may also be untethered to the cell surface and may be cytosolic, nuclear, or released from the cell altogether. Non-cell associated receptors are termed soluble receptors.

All publications or patents cited herein are entirely incorporated herein by reference, whether or not specifically designated accordingly, as they show the state of the art at the time of the present invention and/or provide description and enablement of the present invention. Publications refer to any scientific or patent publications, or any other information available in any media format, including all recorded, electronic or printed formats. The following references are entirely incorporated herein by reference: Ausubel, et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY (1987-2001); Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, NY (1989); Harlow and Lane, antibodies, a Laboratory Manual, Cold Spring Harbor, NY (1989); Colligan, et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY (1997-2001).

Biological Function of Oxytocin Receptor

Novel expression and novel function of the Oxytocin Receptor, and its biological function in airway smooth muscle cells have been identified. The observation originated from data analysis of microarray gene expression profiling of primary human airway smooth muscle cells (HASMC). When compared to HASMC from non-asthmatic subjects, the HASMC from asthmatic subjects consistently showed elevated expression of the Oxytocin Receptor. The observation was confirmed by an independent method – Real Time Polymerase Chain Reaction (PCR) at the messenger RNA level; HASMC expression was up regulated by IL-13 and tumor necrosis factor alpha (TNFα). The Oxytocin Receptor protein was detected in cell lysates from HASMC by immunoblotting.

Furthermore, the expression characteristics of the Oxytocin Receptor were confirmed by functional studies. Sequences coding for the Oxytocin Receptor gene are described herein. The sequences described include nucleic acid sequences of full-length cDNA, open reading frames (ORFs), probes (e.g., for PCR), antisense, ribozymes, and vectors containing the sequences and the polypeptides encoded by them. This demonstrates that the Oxytocin Receptor plays a role in the pathogenesis of an inflammatory disorder, such as asthma, emphysema, chronic obstructive pulmonary disease (COPD), and other pulmonary-

related disorders, and may also play a role in other lung disorders with an inflammatory component.

Such compositions may comprise one or more protein isoforms, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise a cell that expresses Oxytocin Receptor protein, or a T cell that is specific for cells expressing a polypeptide encoded by the gene, or other type of agonists; and antagonistic agents such as neutralizing monoclonal antibodies (mAb) or small molecule compounds to any portion of Oxytocin Receptor DNA, RNA or protein. These compositions may be used, for example, for the prevention and treatment of a range of immue-mediated inflammatory diseases. Diagnostic and prognostic methods based on detecting Oxytocin Receptor protein, or mRNA encoding such a protein, in a sample are disclosed.

Oxytocin and Oxytocin Receptor proteins, polypeptides, and nucleic acid molecules encoding them comprise a family of molecules having certain conserved structural and functional features. Each of these molecules is included in the definition of oxytocin and the Oxytocin Receptor. As used herein, the term "family" is intended to mean two or more proteins or nucleic acid molecules having a common or similar domain structure and having sufficient amino acid or nucleotide sequence identity as defined herein. Family members can be from either the same or a different species. For example, a family can comprise two or more proteins of human origin, or can comprise one or more proteins of human origin and one or more of non-human origin.

A domain that may be present in Oxytocin Receptor proteins is a signal sequence. As used herein, a "signal sequence" includes a peptide of at least about 10 amino acid residues in length which occurs at the amino terminus of membrane-bound proteins and which contains at least about 45% hydrophobic amino acid residues, such as alanine, leucine, isoleucine, phenylalanine, proline, tyrosine, tryptophan, or valine. In a preferred embodiment, a signal sequence contains at least about 10 to 35 amino acid residues, preferably about 10 to 20 amino acid residues, and has at least about 35-60%, more preferably 40-50%, and more preferably at least about 45% hydrophobic residues. A signal sequence serves to direct a protein containing such a sequence to a lipid bilayer. Thus, in one embodiment, an Oxytocin Receptor protein may contain a signal sequence. The signal sequence is cleaved during processing of the mature protein.

Oxytocin Receptor proteins include an extracellular domain. As used herein, an "extracellular domain" refers to a portion of a protein that is localized to the non-cytoplasmic side of a lipid bilayer of a cell when a nucleic acid encoding the protein is expressed in the cell.

The human oxytocin receptor protein extracellular domain is located from amino acid residues 1-38 of SEQ ID NO:2.

In addition, an Oxytocin Receptor includes a transmembrane domain. As used herein, a "transmembrane domain" refers to an amino acid sequence which is at least about 15 amino acid residues in length and which contains at least about 65-70% hydrophobic amino acid residues such as alanine, leucine, phenylalanine, protein, tyrosine, tryptophan, or valine (Erik, et al. Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182). In a preferred embodiment, a transmembrane domain contains about 15-30 amino acid residues, preferably about 20-25 amino acid residues, and has at least about 60-80%, more preferably 65-75%, and more preferably at least about 70% hydrophobic residues. Thus, Oxytocin Receptor protein, has characteristic seven-transmembrane domains which span from residue 39 to residue 332 of the pre-protein of SEQ ID NO:2.

Oxytocin Receptor proteins have a cytoplasmic domain, particularly including proteins having a carboxyl-terminal cytoplasmic domain. As used herein, a "cytoplasmic domain" refers to a portion of a protein that is localized to the cytoplasmic side of a lipid bilayer of a cell when a nucleic acid encoding the protein is expressed in the cell. The human Oxytocin Receptor cytoplasmic domain is situated from amino acid residue 333 to the C-terminus of the protein or residue 389 of SEQ ID NO:2. Oxytocin receptor proteins typically comprise a variety of potential post-translational modification sites (often within an extracellular domain).

OXYTOCIN RECEPTOR Antagonists

As used herein, the term "OXYTOCIN RECEPTOR antagonists" refers to substances which inhibit or neutralize the biologic activity of OXYTOCIN RECEPTOR. Such antagonists accomplish this effect in a variety of ways. One class of OXYTOCIN RECEPTOR antagonists will bind to the OXYTOCIN RECEPTOR protein with sufficient affinity and specificity to neutralize the biologic effects of the OXYTOCIN RECEPTOR. Included in this class of molecules are antibodies and antibody fragments (such as, for example, F(ab) or $F(ab')_2$ molecules). Another class of OXYTOCIN RECEPTOR antagonists comprises fragments of the OXYTOCIN RECEPTOR protein, muteins or small organic molecules, i.e., peptidomimetics, that will bind to the OXYTOCIN RECEPTOR or OXYTOCIN RECEPTOR binding partners, thereby inhibiting the biologic activity of the OXYTOCIN RECEPTOR. The OXYTOCIN RECEPTOR antagonist may be of any of these classes as long as it is a substance that inhibits OXYTOCIN RECEPTOR biologic activity. OXYTOCIN RECEPTOR antagonists include OXYTOCIN RECEPTOR antibody, oxytocin antibody, modified OXYTOCIN RECEPTOR, and partial

peptides of the OXYTOCIN RECEPTOR. Another class of OXYTOCIN RECEPTOR antagonists include siRNAs, shRNAs, antisense molecules and DNAzymes targeting the OXYTOCIN RECEPTOR gene sequence as known in the art are disclosed herein.

Accordingly, as used herein, an "OXYTOCIN RECEPTOR antibody," "anti-OXYTOCIN RECEPTOR antibody," "anti-OXYTOCIN RECEPTOR antibody portion," or "anti-OXYTOCIN RECEPTOR antibody fragment" and/or "anti-OXYTOCIN RECEPTOR antibody variant" and the like include any protein or polypeptide containing molecule that comprises at least a portion of an immunoglobulin molecule, such as but not limited to at least one complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or any portion thereof, or at least one portion of an Oxytocin Receptor binding protein derived from an Oxytocin Receptor protein or peptide, which can be incorporated into an antibody for use in the present invention. Such antibody optionally further affects a specific ligand, such as, but not limited to, where such antibody modulates, decreases, increases, antagonizes, agonizes, mitigates, alleviates, blocks, inhibits, abrogates and/or interferes with Oxytocin Receptor activity, in vitro, in situ and/or in vivo. As a non-limiting example, a suitable anti-Oxytocin Receptor antibody, specified portion or variant of the present invention can bind at least one Oxytocin Receptor protein or peptide, or specified portions, variants or domains thereof. A suitable anti-OXYTOCIN RECEPTOR antibody, specified portion, or variant affects Oxytocin Receptor angiogenic function in a variety of ways, such as, but not limited to, RNA, DNA or protein synthesis, Oxytocin Receptor release, Oxytocin Receptor signaling, Oxytocin Receptor binding, Oxytocin Receptor production and/or synthesis.

Antibodies can include one or more of at least one CDR, at least one variable region, at least one constant region, at least one heavy chain (e.g., g1, g2, g3, g4, m, a1, a2, d, e), at least one light chain (e.g., k and l), or any portion or fragment thereof, and can further comprise interchain and intrachain disulfide bonds, hinge regions, glycosylation sites that can be separated by a hinge region, as well as heavy chains and light chains. Light chains typically have a molecular weight of about 25Kd and heavy chains typically range from about 50K-77Kd. Light chains can exist in two distinct forms or isotypes, kappa (k) and lambda (l), which can combine with any of the heavy chain types. All light chains have at least one variable region and at least one constant region. The IgG antibody is considered a typical antibody structure and has two intrachain disulfide bonds in the light chain (one in the variable region and one in the constant region), with four in the heavy chain, and such bond encompassing a peptide loop of about 60-70 amino acids comprising a "domain" of about 110 amino acids in the chain. IgG antibodies can be characterized into four classes, IgG1, IgG2, IgG3 and IgG4. Each

immunoglobulin class has a different set of functions. The following table summarizes the Physicochemical properties of each of the immunoglobulin classes and subclasses.

Property	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	SigA	IgD	IgE
Heavy Chain	γ1	γ1	γ1	γ1	μ	α1	α2	α1 /α2	δ	e
Mean Serum conc. (mg/ml)	9	3	1	0.5	1.5	3.0	0.5	0.05	0.03	0.00 05
Sedimentation constant	7s	7s	7s	7s	19s	7s	7s	11s	7s	8s
Mol. Wt. (X 10^3)	146	146	170	146	970	160	160	385	184	188
Half Life (days)	21	20	7	21	10	6	6	?	3	2
% intravascular distribution	45	45	45	45	80	42	42	Trace	75	50
Carbohydrate (%)	2-3	2-3	2-3	2-3	12	7-11	7-11	7-11	9-14	12

5 The following table summarizes non-limiting examples of antibody effector functions for human antibody classes and subclasses.

Effector function	IgG1	IgG2	IgG3	IgG4	IgM	IgA	IgD	IgE
Complement fixation	+	+/-	++	-	++	-	-	-
Placental transfer	+	+/-	+	+	-	-	-	-
Binding to Staph A	+++	+++	-	+++	-	-	-	-
Binding to Strep G	+++	+++	+++	+++	-	-	-	-

+++ = very high; ++ = high; + = moderate; +/- = minimal; - = none; ? = questionable

Accordingly, the type of antibody or fragment thereof can be selected for use 10 according to the present invention based on the desired characteristics and functions that are desired for a particular therapeutic or diagnostic use, such as but not limited to, serum half life, intravascular distribution, complement fixation, etc.

An isolated Oxytocin Receptor or oxytocin polypeptide, or a fragment thereof, can be used as an immunogen to generate antibodies using standard techniques for polyclonal 15 and monoclonal antibody preparation. The full-length polypeptide or protein can be used or, alternatively, the invention provides antigenic peptide fragments for use as immunogens. The antigenic peptide of an Oxytocin Receptor or oxytocin protein comprises at least 8 (preferably 10, 15, 20, or 30 or more) amino acid residues and encompasses an epitope of the protein such that an antibody raised against the peptide forms a specific immune complex with the protein.

20 An immunogen typically is used to prepare antibodies by immunizing a suitable (i.e., immunocompetent) subject, such as a rabbit, goat, mouse, or other mammal or vertebrate.

An appropriate immunogenic preparation can contain, for example, recombinantly expressed or chemically synthesized polypeptide. The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent.

Antibody-producing cells can be obtained from the peripheral blood or, preferably, the spleen or lymph nodes of humans or other suitable animals that have been immunized with the immunogen of interest. Any other suitable host cell can also be used for expressing heterologous or endogenous nucleic acid encoding an antibody, specified fragment or variant thereof, of the present invention. The fused cells (hybridomas) or recombinant cells can be isolated using selective culture conditions or other suitable known methods, and cloned by limiting dilution or cell sorting, or other known methods. Cells that produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

In one approach, a hybridoma is produced by fusing a suitable immortal cell line (e.g., a myeloma cell line, such as, but not limited to, Sp2/0, Sp2/0-AG14, NSO, NS1, NS2, AE-1, L.5, >243, P3X63Ag8.653, Sp2 SA3, Sp2 MAI, Sp2 SS1, Sp2 SA5, U937, MLA 144, ACT IV, MOLT4, DA-1, JURKAT, WEHI, K-562, COS, RAJI, NIH 3T3, HL-60, MLA 144, NAMALWA, NEURO 2A, or the like), or heteromyelomas, fusion products thereof, or any cell or fusion cell derived therefrom, or any other suitable cell line as known in the art (see, e.g., www.atcc.org, www.lifetech.com, and the like), with antibody producing cells, such as, but not limited to, isolated or cloned spleen, peripheral blood, lymph, tonsil, or other immune or B cell containing cells, or any other cells expressing heavy or light chain constant or variable or framework or CDR sequences, either as endogenous or heterologous nucleic acid, as recombinant or endogenous, viral, bacterial, algal, prokaryotic, amphibian, insect, reptilian, fish, mammalian, rodent, equine, ovine, goat, sheep, primate, eukaryotic, genomic DNA, cDNA, rDNA, mitochondrial DNA or RNA, chloroplast DNA or RNA, hnRNA, mRNA, tRNA, single, double or triple stranded, hybridized, and the like or any combination thereof. See, e.g., Ausubel, supra, and Colligan, Immunology, supra, chapter 2, entirely incorporated herein by reference.

Other suitable methods of producing or isolating antibodies of the requisite specificity can be used, including, but not limited to, methods that select recombinant antibody from a peptide or polypeptide library (e.g., but not limited to, a bacteriophage, ribosome, oligonucleotide, RNA, cDNA, or the like, display library; e.g., as available from Cambridge antibody Technologies, Cambridgeshire, UK; MorphoSys, Martinsreid/Planegg, DE; Biovation, Aberdeen, Scotland, UK; BioInvent, Lund, Sweden; Dyax Corp., Enzon, Affymax/Biosite; Xoma, Berkeley, CA; Ixsys. See, e.g., EP Publication No. 368,684, PCT/GB91/01134; PCT/GB92/01755; PCT/GB92/002240; PCT/GB92/00883; PCT/GB93/00605; U.S. Pat.

5,962,255; PCT/GB94/01422; PCT/GB94/02662; PCT/GB97/01835; (CAT/MRC); WO90/14443; WO90/14424; WO90/14430; PCT/US94/1234; WO92/18619; WO96/07754; (Scripps); EP 614 989 (MorphoSys); WO95/16027 (BioInvent); WO88/06630; WO90/3809 (Dyax); US 4,704,692 (Enzon); PCT/US91/02989 (Affymax); WO89/06283; EP 371 998; EP 550 400; (Xoma); EP 229 046; PCT/US91/07149 (Ixsys); or stochastically generated peptides or polypeptides – U.S. Pat. 5723323, 5763192, 5814476, 5817483, 5824514, and 5976862, WO 86/05803, EP 590 689 (Ixsys, now Applied Molecular Evolution (AME), each entirely incorporated herein by reference) or that rely upon immunization of transgenic animals (e.g., SCID mice, Nguyen et al., Microbiol. Immunol. 41:901-907 (1997); Sandhu et al., Crit. Rev. Biotechnol. 16:95-118 (1996); Eren et al., Immunol. 93:154-161 (1998), each entirely incorporated by reference as well as related patents and applications) that are capable of producing a repertoire of human antibodies, as known in the art and/or as described herein. Such techniques, include, but are not limited to, ribosome display (Hanes et al., Proc. Natl. Acad. Sci. USA, 94:4937-4942 (May 1997); Hanes et al., Proc. Natl. Acad. Sci. USA, 95:14130-14135 (Nov. 1998)); single cell antibody producing technologies (e.g., selected lymphocyte antibody method ("SLAM") (U.S. Pat. No. 5,627,052, Wen et al., J. Immunol. 17:887-892 (1987); Babcook et al., Proc. Natl. Acad. Sci. USA 93:7843-7848 (1996)); gel microdroplet and flow cytometry (Powell et al., Biotechnol. 8:333-337 (1990); One Cell Systems, Cambridge, MA; Gray et al., J. Imm. Meth. 182:155-163 (1995); Kenny et al., Bio/Technol. 13:787-790 (1995)); B-cell selection (Steenbakkers et al., Molec. Biol. Reports 19:125-134 (1994); Jonak et al., Progress Biotech, Vol. 5, In Vitro Immunization in Hybridoma Technology, Borrebaeck, ed., Elsevier Science Publishers B.V., Amsterdam, Netherlands (1988)).

Methods for engineering or humanizing non-human or human antibodies can also be used and are well known in the art. Generally, a humanized or engineered antibody has one or more amino acid residues from a source that is non-human, e.g., but not limited to, mouse, rat, rabbit, non-human primate or other mammal. These human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable, constant or other domain of a known human sequence. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez/query.fcgi; www.ncbi.nih.gov/igblast; www.atcc.org/phage/hdb.html; www.mrc-cpe.cam.ac.uk/ALIGNMENTS.php; www.kabatdatabase.com/top.html; ftp.ncbi.nih.gov/repository/kabat; www.sciquest.com; www.abcam.com; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/~pedro/research_tools.html; www.whfreeman.com/immunology/CH05/kuby05.htm; www.hhmi.org/grants/lectures/1996/vlab; www.path.cam.ac.uk/~mrc7/mikeimages.html; mcb.harvard.edu/BioLinks/Immunology.html;

www.immunologylink.com; pathbox.wustl.edu/~hcenter/index.html; www.appliedbiosystems.com; www.nal.usda.gov/awic/pubs/antibody; www.m.ehime-u.ac.jp/~yasuhito/Elisa.html; www.biodesign.com; www.cancerresearchuk.org; www.biotech.ufl.edu; www.isac-net.org; baserv.uci.kun.nl/~jraats/links1.html; www.recab.uni-hd.de/immuno.bme.nwu.edu; www.mrc-cpe.cam.ac.uk; www.ibt.unam.mx/vir/V_mice.html; http://www.bioinf.org.uk/abs; antibody.bath.ac.uk; www.unizh.ch; www.cryst.bbk.ac.uk/~ubcg07s; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.html; www.path.cam.ac.uk/~mrc7/humanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.jerini.de; Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference.

Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art. Generally, part or all of the non-human or human CDR sequences are maintained while the non-human sequences of the variable and constant regions are replaced with human or other amino acids. Antibodies can also optionally be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, humanized antibodies can be optionally prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Humanization or engineering of antibodies of the present invention can be performed using any known method, such as but not limited to, those described in, Winter (Jones et al., Nature 321:522 (1986); Riechmann et al., Nature 332:323 (1988); Verhoeyen et al., Science 239:1534 (1988)), Sims et al., J. Immunol. 151: 2296 (1993); Clothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), U.S. Patent Nos: 5723323; 5976862; 5824514; 5817483; 5814476; 5763192; 5723323; 5766886;

5714352; 6204023; 6180370; 5693762; 5530101; 5585089; 5225539; and 4816567; PCT/: US98/16280; US96/18978; US91/09630; US91/05939; US94/01234; GB89/01334; GB91/01134; GB92/01755; WO90/14443; WO90/14424; and WO90/14430; EP 229246; each entirely incorporated herein by reference, including references cited therein.

5 The OXYTOCIN RECEPTOR antibody can also be optionally generated by immunization of a transgenic animal (e.g., mouse, rat, hamster, non-human primate, and the like) capable of producing a repertoire of human antibodies, as described herein and/or as known in the art. Cells that produce a human OXYTOCIN RECEPTOR antibody can be isolated from such animals and immortalized using suitable methods, such as the methods 10 described herein.

Transgenic mice that can produce a repertoire of human antibodies that bind to human antigens can be produced by known methods (e.g., but not limited to, U.S. Pat. Nos: 5,770,428, 5,569,825, 5,545,806, 5,625,126, 5,625,825, 5,633,425, 5,661,016 and 5,789,650 issued to Lonberg *et al.*; Jakobovits *et al.* WO 98/50433, Jakobovits *et al.* WO 98/24893, 15 Lonberg *et al.* WO 98/24884, Lonberg *et al.* WO 97/13852, Lonberg *et al.* WO 94/25585, Kucherlapate *et al.* WO 96/34096, Kucherlapate *et al.* EP 0463 151 B1, Kucherlapate *et al.* EP 0710 719 A1, Surani *et al.* US. Pat. No. 5,545,807, Bruggemann *et al.* WO 90/04036, Bruggemann *et al.* EP 0438 474 B1, Lonberg *et al.* EP 0814 259 A2, Lonberg *et al.* GB 2 272 440 A, Lonberg *et al.* *Nature* 368:856-859 (1994), Taylor *et al., Int. Immunol.* 6(4)579-591 20 (1994), Green *et al, Nature Genetics* 7:13-21 (1994), Mendez *et al., Nature Genetics* 15:146-156 (1997), Taylor *et al., Nucleic Acids Research* 20(23):6287-6295 (1992), Tuaillon *et al., Proc Natl Acad Sci USA* 90(8)3720-3724 (1993), Lonberg *et al., Int Rev Immunol* 13(1):65-93 (1995) and Fishwald *et al., Nat Biotechnol* 14(7):845-851 (1996), which are each entirely incorporated herein by reference). Generally, these mice comprise at least one transgene comprising DNA 25 from at least one human immunoglobulin locus that is functionally rearranged, or which can undergo functional rearrangement. The endogenous immunoglobulin loci in such mice can be disrupted or deleted to eliminate the capacity of the animal to produce antibodies encoded by endogenous genes.

Antibodies of the present invention can also be prepared in milk by 30 administering at least one anti-OXYTOCIN RECEPTOR antibody encoding nucleic acid to transgenic animals or mammals, such as goats, cows, horses, sheep, and the like, that produce antibodies in their milk. Such animals can be provided using known methods. See, e.g., but not limited to, U.S. Patent Nos. 5,827,690; 5,849,992; 4,873,316; 5,849,992; 5,994,616; 5,565,362; 5,304,489, and the like, each of which is entirely incorporated herein by reference.

Antibodies of the present invention can additionally be prepared using at least one OXYTOCIN RECEPTOR antibody encoding nucleic acid to provide transgenic plants and cultured plant cells (e.g., but not limited to, tobacco and maize) that produce such antibodies, specified portions or variants in the plant parts or in cells cultured therefrom.

5 The antibodies of the invention can bind human Oxytocin Receptor with a wide range of affinities (K_D). In a preferred embodiment, at least one human mAb of the present invention can optionally bind human Oxytocin Receptor with high affinity. For example, a human mAb can bind human Oxytocin Receptor with a K_D equal to or less than about 10^{-7} M, such as but not limited to, 0.1-9.9 (or any range or value therein) X 10^{-7}, 10^{-8}, 10^{-9},10^{-10}, 10^{-11},
10 10^{-12}, 10^{-13} or any range or value therein.

The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method. (See, for example, Berzofsky, *et al.*, "Antibody-Antigen Interactions," In *Fundamental Immunology*, Paul, W. E., Ed., Raven Press: New York, NY (1984); Kuby, Janis *Immunology*, W. H. Freeman and Company: New York, NY (1992); and
15 methods described herein). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions (e.g., salt concentration, pH). Thus, measurements of affinity and other antigen-binding parameters (e.g., K_D, K_a, K_d) are preferably made with standardized solutions of antibody and antigen, and a standardized buffer, such as the buffer described herein.

20 An OXYTOCIN RECEPTOR Antagonist (e.g., monoclonal antibody) can be used to isolate the Oxytocin Receptor polypeptide by standard techniques, such as affinity chromatography or immunoprecipitation. Moreover, such an antibody can be used to detect the protein (e.g., in a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the polypeptide. The antibodies can also be used diagnostically to
25 monitor protein levels in tissue as part of a clinical testing procedure, e.g., to, for example, determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, and radioactive materials. Examples of suitable enzymes include horseradish
30 peroxidase, alkaline phosphatase, β-galactosidase, and acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a

luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin, and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{35}S or ^{3}H.

The term "antibody" is further intended to encompass antibodies, digestion fragments, specified portions and variants thereof, including antibody mimetics or comprising
5 portions of antibodies that mimic the structure and/or function of an antibody or specified fragment or portion thereof, including single chain antibodies and fragments thereof. Functional fragments include antigen-binding fragments that bind to a mammalian Oxytocin Receptor. For example, antibody fragments capable of binding to the Oxytocin Receptor or portions thereof, including, but not limited to, Fab (e.g., by papain digestion), Fab' (e.g., by pepsin digestion and
10 partial reduction) and F(ab')2 (e.g., by pepsin digestion), facb (e.g., by plasmin digestion), pFc' (e.g., by pepsin or plasmin digestion), Fd (e.g., by pepsin digestion, partial reduction and reaggregation), Fv or scFv (e.g., by molecular biology techniques) fragments, are encompassed by the invention (see, e.g., Colligan, Immunology, *supra*).

Such fragments can be produced by enzymatic cleavage, synthetic or
15 recombinant techniques, as known in the art and/or as described herein. Antibodies can also be produced in a variety of truncated forms using antibody genes in which one or more stop codons have been introduced upstream of the natural stop site. For example, a combination gene encoding a F(ab')2 heavy chain portion can be designed to include DNA sequences encoding the C_H1 domain and/or hinge region of the heavy chain. The various portions of
20 antibodies can be joined together chemically by conventional techniques, or can be prepared as a contiguous protein using genetic engineering techniques.

The anti-OXYTOCIN RECEPTOR antibody may be a primate, rodent, or human antibody or a chimeric or humanized antibody. As used herein, the term "human antibody" refers to an antibody in which substantially every part of the protein (e.g., CDR, framework, CL,
25 CH domains (e.g., C_H1, C_H2, C_H3), hinge, (VL, VH)) is substantially non-immunogenic in humans, with only minor sequence changes or variations, and/or is engineered to, derived from, or contains known human antibody components. Similarly, antibodies designated primate (monkey, baboon, chimpanzee, etc.), rodent (mouse, rat, rabbit, guinea pig, hamster, and the like) and other mammals designate such species, sub-genus, genus, sub-family, family specific
30 antibodies. Further, chimeric antibodies of the invention can include any combination of the above. Such changes or variations optionally and preferably retain or reduce the immunogenicity in humans or other species relative to non-modified antibodies. Thus, a human antibody is distinct from a chimeric or humanized antibody. It is pointed out that a human antibody can be produced by a non-human animal or prokaryotic or eukaryotic cell that is

capable of expressing functionally rearranged human immunoglobulin (e.g., heavy chain and/or light chain) genes. Further, when a human antibody is a single chain antibody, it can comprise a linker peptide that is not found in native human antibodies. For example, a Fv can comprise a linker peptide, such as 2 to about 8 glycine or other amino acid residues, which connects the variable region of the heavy chain and the variable region of the light chain. Such linker peptides are considered to be of human origin.

Bispecific, heterospecific, heteroconjugate or similar antibodies can also be used that are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for at least one Oxytocin Receptor protein, the other one is for any other antigen, e.g., oxytocin. Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed, e.g., in WO 93/08829, US Patent Nos, 6210668, 6193967, 6132992, 6106833, 6060285, 6037453, 6010902, 5989530, 5959084, 5959083, 5932448, 5833985, 5821333, 5807706, 5643759, 5601819, 5582996, 5496549, 4676980, WO 91/00360, WO 92/00373, EP 03089, Traunecker et al., EMBO J. 10:3655 (1991), Suresh et al., Methods in Enzymology 121:210 (1986), each entirely incorporated herein by reference.

Anti-OXYTOCIN RECEPTOR antibodies useful in the methods and compositions of the present invention can optionally be characterized by high affinity binding to the Oxytocin Receptor and optionally and preferably having low toxicity. In particular, an antibody, specified fragment or variant of the invention, where the individual components, such as the variable region, constant region and framework, individually and/or collectively, optionally and preferably possess low immunogenicity, is useful in the present invention. The antibodies that can be used in the invention are optionally characterized by their ability to treat patients for extended periods with measurable alleviation of symptoms and low and/or acceptable toxicity. Low or acceptable immunogenicity and/or high affinity, as well as other suitable properties, can contribute to the therapeutic results achieved. "Low immunogenicity" is defined herein as raising significant HAHA, HACA or HAMA responses in less than about 75%, or preferably less than about 50% of the patients treated and/or raising low titres in the patient treated (less than

about 300, preferably less than about 100 measured with a double antigen enzyme immunoassay) (Elliott et al., Lancet 344:1125-1127 (1994), entirely incorporated herein by reference).

Suitable antibodies include those that compete for binding to human Oxytocin Receptor with monoclonal antibodies that block Oxytocin Receptor activation.

OXYTOCIN RECEPTOR Antagonists in the form of siRNA, shRNA, and DNAzymes

A therapeutic targeting the inducer of the Oxytocin Receptor may provide better chances of success. Gene expression can be modulated in several different ways including by the use of siRNAs, shRNAs, antisense molecules and DNAzymes. Synthetic siRNAs, shRNAs, and DNAzymes can be designed to specifically target one or more genes and they can easily be delivered to cells in vitro or in vivo.

The present invention encompasses antisense nucleic acid molecules, i.e., molecules that are complementary to a sense nucleic acid encoding an Oxytocin Receptor polypeptide, e.g., complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof, e.g., all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding an Oxytocin Receptor polypeptide. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences that flank the coding region and are not translated into amino acids.

An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives, peptide nucleic acids (PNAs), and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5- chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5- (carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine,

2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D- mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2- thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6- diaminopurine, as well as shown in the table below. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been sub-cloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

The antisense nucleic acid molecules of the invention are typically administered to a subject or generated in situ such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a selected Oxytocin Receptor polypeptide to thereby inhibit expression, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then be administered systemically. For example, for systemic administration, antisense molecules can be modified such that they specifically bind to receptors or antigens expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong pol II or pol III promoter are preferred.

An antisense nucleic acid molecule of the invention can be an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual α-units, the strands run parallel to each other (Gaultier et al. (1987) *Nucleic Acids Res.* 15:6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al. (1987) *Nucleic Acids Res.* 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al. (1987) *FEBS Lett.* 215:327-330).

The invention also encompasses ribozymes. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which they have a complementary region. Thus, ribozymes (e.g., hammerhead ribozymes as described in Haselhoff and Gerlach (1988) *Nature* 334:585-591) can be used to catalytically cleave mRNA transcripts to thereby inhibit translation of the protein encoded by the mRNA. A ribozyme having specificity for a nucleic acid molecule encoding an Oxytocin Receptor polypeptide can be designed based upon the nucleotide sequence of a cDNA disclosed herein. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in Cech et al., U.S. Pat. No. 4,987,071; and Cech et al., U.S. Pat. No. 5,116,742. Alternatively, an mRNA encoding an Oxytocin Receptor polypeptide can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Haselhoff and Gerlach supra; Bartel and Szostak (1993) *Science* 261:1411-1418.

The invention also encompasses ribonucleic acid molecules which are complementary, antisense, double stranded homologues, siRNA, or are sequence specific single-stranded RNAs which form short hairpin structures, shRNA (collectively, interfering RNA), that can be used to down-modulate specific gene expression, in this case, an Oxytocin Receptor, and therefore to inhibit protein expression and to elucidate their respective biological functions. (Fire, A., et al. (1998) Nature 391: 806-811; Paddison, P.J. et al. (2002) Genes Develop 16:948-958).

In various embodiments, the nucleic acid molecules of the invention can be modified at the base moiety, sugar moiety, or phosphate backbone to improve, e.g., the stability, hybridization, or solubility of the molecule. For example, the nucleotide analogs shown in the table below and as described above, can be substituted for the naturally occurring nucleotides.

In another example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup et al. (1996) *Bioorganic & Medicinal Chemistry* 4(1): 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, e.g., DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed

using standard solid phase peptide synthesis protocols as described in Hyrup et al. (1996), supra; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 14670-675.

PNAs can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene
5 expression by, e.g., inducing transcription or translation arrest or inhibiting replication. PNAs can also be used, e.g., in the analysis of single base pair mutations in a gene by, e.g., PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, e.g., S1 nucleases (Hyrup (1996), supra; or as probes or primers for DNA sequence and hybridization (Hyrup (1996), supra; Perry-O'Keefe et al. (1996) *Proc. Natl. Acad. Sci. USA*
10 93: 14670-675).

In another embodiment, PNAs can be modified, e.g., to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated which can combine the
15 advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, e.g., RNASE H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996), supra). The synthesis of PNA-DNA chimeras can
20 be performed as described in Hyrup (1996), supra, and Finn et al. (1996) *Nucleic Acids Res.* 24(17): 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry and modified nucleoside analogs. Compounds, such as 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used as a link between the PNA and the 5' end of DNA (Mag et al. (1989) *Nucleic Acids Res.* 17:5973-88).
25 PNA monomers are then coupled in a step-wise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn et al. (1996) *Nucleic Acids Res.* 24(17):3357-63). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment (Peterser et al. (1975) *Bioorganic Med. Chem. Lett.* 5:1119-11124).

In other embodiments, the oligonucleotide can include other appended groups,
30 such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:6553-6556; Lemaitre et al. (1987) *Proc. Natl. Acad. Sci. USA* 84:648-652; PCT Publication No. WO 88/09810) or the blood-brain barrier (see, e.g., PCT Publication No. WO 89/10134). In addition, oligonucleotides can be modified with hybridization-triggered cleavage agents (see, e.g., Krol et

al. (1988) *Bio/Techniques* 6:958-976) or intercalating agents (see, e.g., Zon (1988) *Pharm. Res.* 5:539-549). To this end, the oligonucleotide can be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

5 Proteins

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises all or part (preferably biologically active) of an Oxytocin Receptor polypeptide operably linked to a heterologous polypeptide (i.e., a polypeptide other than the same Oxytocin Receptor polypeptide). Within the fusion protein, the term 10 "operably linked" is intended to indicate that the Oxytocin Receptor polypeptide and the heterologous polypeptide are fused in-frame to each other. The heterologous polypeptide can be fused to the amino-terminus or the carboxyl-terminus of the Oxytocin Receptor polypeptide. In another embodiment, an Oxytocin Receptor polypeptide or a domain or active fragment thereof can be fused with a heterologous protein sequence or fragment thereof to form a 15 chimeric protein, where the polypeptides, domains or fragments are not fused end to end but are interposed within the heterologous protein framework.

One useful fusion protein is a GST fusion protein in which the Oxytocin Receptor polypeptide is fused to the carboxyl terminus of GST sequences. Such fusion proteins can facilitate the purification of a recombinant Oxytocin Receptor polypeptide.

20 In another embodiment, the fusion protein contains a heterologous signal sequence at its amino terminus. For example, the native signal sequence of an Oxytocin Receptor polypeptide can be removed and replaced with a signal sequence from another protein. For example, the gp67 secretory sequence of the baculovirus envelope protein can be used as a heterologous signal sequence (*Current Protocols in Molecular Biology*, Ausubel et al., 25 eds., John Wiley & Sons, 1992). Other examples of eukaryotic heterologous signal sequences include the secretory sequences of melittin and human placental alkaline phosphatase (Stratagene; La Jolla, Calif.). In yet another example, useful prokaryotic heterologous signal sequences include the phoA secretory signal (Sambrook et al., supra) and the protein A secretory signal (Pharmacia Biotech; Piscataway, N.J.).

30 In yet another embodiment, the fusion protein is an immunoglobulin fusion protein in which all or part of an Oxytocin Receptor polypeptide is fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a ligand (soluble or membrane-bound) and a protein on the

surface of a cell (receptor), to thereby suppress signal transduction in vivo. The immunoglobulin fusion protein can be used to affect the bioavailability of a cognate ligand of an Oxytocin Receptor polypeptide. Inhibition of ligand/receptor interaction can be useful therapeutically, both for treating proliferative and differentiative disorders and for modulating
5 (e.g., promoting or inhibiting) cell survival. A preferred embodiment of an immunoglobulin chimeric protein is a C_H1 domain-deleted immunoglobulin or "mimetibody" having an active polypeptide fragment interposed within a modified framework region as taught in co-pending application PCT WO/04002417. Moreover, the immunoglobulin fusion proteins of the invention can be used as immunogens to produce antibodies directed against an Oxytocin Receptor
10 polypeptide in a subject, to purify ligands and in screening assays to identify molecules that inhibit the interaction of receptors with ligands.

Chimeric and fusion proteins of the invention can be produced by standard recombinant DNA techniques. In another embodiment, the fusion gene can be synthesized by conventional techniques, including automated DNA synthesizers. Alternatively, PCR
15 amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and re-amplified to generate a chimeric gene sequence (see, e.g., Ausubel et al., supra). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A nucleic acid encoding an Oxytocin Receptor
20 polypeptide can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the Oxytocin Receptor polypeptide.

A signal sequence of an Oxytocin Receptor polypeptide can be used to facilitate secretion and isolation of the secreted protein or other proteins of interest. Signal sequences are typically characterized by a core of hydrophobic amino acids that are generally
25 cleaved from the mature protein during secretion in one or more cleavage events. Such signal peptides contain processing sites that allow cleavage of the signal sequence from the mature proteins as they pass through the secretory pathway. Thus, the invention pertains to the described polypeptides having a signal sequence, as well as to the signal sequence itself and to the polypeptide in the absence of the signal sequence (i.e., the cleavage products). In one
30 embodiment, a nucleic acid sequence encoding a signal sequence of the invention can be operably linked in an expression vector to a protein of interest, such as a protein that is ordinarily not secreted or is otherwise difficult to isolate. The signal sequence directs secretion of the protein, such as from a eukaryotic host into which the expression vector is transformed, and the signal sequence is subsequently or concurrently cleaved. The protein can then be
35 readily purified from the extracellular medium by art recognized methods. Alternatively, the

signal sequence can be linked to the protein of interest using a sequence that facilitates purification, such as with a GST domain.

In another embodiment, the signal sequences of the present invention can be used to identify regulatory sequences, e.g., promoters, enhancers, and/or repressors. Since signal sequences are the most amino-terminal sequences of a peptide, the nucleic acids flanking the signal sequence on its amino-terminal side are likely regulatory sequences that affect transcription. Thus, a nucleotide sequence that encodes all or a portion of a signal sequence can be used as a probe to identify and isolate signal sequences and their flanking regions, and these flanking regions can be studied to identify regulatory elements therein.

The present invention also pertains to variants of the Oxytocin Receptor polypeptides and can include one or more amino acid substitutions, deletions or additions, either from natural mutations or human manipulation, as specified herein. Such mutations or substitutions can include muteins, whose mutations can be significant enough to alter the properties of the peptide without altering the biological activity of the peptide to inhibit the binding of human Oxytocin Receptor to its ligand. Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. In certain embodiments of the invention, the number of amino acid substitutions, insertions or deletions for any given Oxytocin Receptor polypeptide, fragment or variant will not be more than 1-5, or any range or value therein, as specified herein.

The Oxytocin Receptor polypeptides may also comprise modified, non-naturally occurring and unusual amino acids substituted or added to their amino acid sequences. A list of exemplary modified, non-naturally occurring and unusual amino acids is provided below.

Modified (Unusual) Amino Acid	**Symbol**
2-Aminoadipic acid	Aad
3-Aminoadipic acid	Baad
beta-Alanine, beta-Aminopropionic acid	bAla
2-Aminobutyric acid	Abu
4-Aminobutyric acid, piperidinic acid	4Abu
6-Aminocaproic acid	Acp

Modified (Unusual) Amino Acid	Symbol
2-Aminoheptanoic acid	Ahe
2-Aminoisobutyric acid	Aib
3-Aminoisobutyric acid	BAib
2-Aminopimelic acid	Apm
2,4-Diaminobutyric acid	Dbu
Desmosine	Des
2,2' -Diaminopimelic acid	Dpm
2,3-Diaminopropionic acid	Dpr
N-Ethylglycine	EtGly
N-Ethylasparagine	EtAsn
Hydroxylysine	Hyl
allo-Hydroxylysine	AHyl
3-Hydroxyproline	3Hyp
4-Hydroxyproline	4Hyp
Isodesmosine	Ide
allo-Isoleucine	AIle
N-Methylglycine, sarcosine	MeGly
N-Methylisoleucine	MeIle
6-N-Methyllysine	MeLys
N-Methylvaline	MeVal

Modified (Unusual) Amino Acid	Symbol
Norvaline	Nva
Norleucine	Nle
Ornithine	Orn

Amino acids in an Oxytocin Receptor polypeptide that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (e.g., Ausubel, *supra*, Chapters 8, 15; Cunningham and Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity, such as, but not limited to, at least one Oxytocin Receptor neutralizing activity.

Such variants have an altered amino acid sequence and can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, e.g., discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade that includes the protein of interest. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the protein.

Variants of an Oxytocin Receptor polypeptide that function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the protein of the invention for agonist or antagonist activity. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential protein sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (e.g., for phage display). There are a variety of methods that can be used to produce libraries of potential variants of the Oxytocin Receptor polypeptide from a degenerate oligonucleotide sequence.

Methods for synthesizing degenerate oligonucleotides are known in the art (see, e.g., Narang (1983) *Tetrahedron* 39:3; Itakura et al. (1984) *Annu. Rev. Biochem.* 53:323; Itakura et al. (1984) *Science* 198:1056; Ike et al. (1983) *Nucleic Acid Res.* 11:477).

In addition, libraries of fragments of the coding sequence of an Oxytocin
5 Receptor polypeptide can be used to generate a variegated population of polypeptides for screening and subsequent selection of variants. For example, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of the coding sequence of interest with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded
10 DNA which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes amino terminal and internal fragments of various sizes of the protein of interest.

15 Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with
20 the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a technique which enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify variants of an Oxytocin Receptor Antagonist polypeptide (Arkin and
25 Yourvan (1992) *Proc. Natl. Acad. Sci. USA* 89:7811-7815; Delgrave et al. (1993) *Protein Engineering* 6(3):327-331).

Compositions and Their Uses

In accordance with the invention, the neutralizing anti-OXYTOCIN RECEPTOR antagonists, such as monoclonal antibodies, described herein can be used to inhibit Oxytocin
30 Receptor activity. Additionally, such antagonists can be used to inhibit Oxytocin Receptor-related inflammatory diseases amenable to such treatment, which may include, but are not limited to, pulmonary-related disorders. The individual to be treated may be any mammal and is preferably a primate, a companion animal which is a mammal and most preferably a human

patient. The amount of antagonist administered will vary according to the purpose it is being used for and the method of administration.

The anti-OXYTOCIN RECEPTOR antagonists may be administered by any number of methods that result in an effect in tissue in which Oxytocin Receptor activity is
5 desired to be prevented or halted. Further, the anti-OXYTOCIN RECEPTOR antagonists need not be present locally to impart an effect on the Oxytocin Receptor activity, therefore, they may be administered wherever access to body compartments or fluids containing Oxytocin Receptor is achieved. In the case of inflamed, malignant, or otherwise compromised tissues, these methods may include direct application of a formulation containing the antagonists. Such
10 methods include intravenous administration of a liquid composition, transdermal administration of a liquid or solid formulation, oral, topical administration, or interstitial or inter-operative administration. Adminstration may be affected by the implantation of a device whose primary function may not be as a drug delivery vehicle.

Administration may also be oral or by local injection into a tumor or tissue but
15 generally, a monoclonal antibody is administered intravenously. Generally, the dosage range is from about 0.05 mg/kg to about 12.0 mg/kg. This may be as a bolus or as a slow or continuous infusion which may be controlled by a microprocessor controlled and programmable pump device.

Alternatively, DNA encoding preferably a fragment of a monoclonal antibody
20 may be isolated from hybridoma cells and administered to a mammal. The DNA may be administered in naked form or inserted into a recombinant vector, e.g., vaccinia virus, in a manner which results in expression of the DNA in the cells of the patient and delivery of the antibody.

The monoclonal antibody used in the method of the present invention may be
25 formulated by any of the established methods of formulating pharmaceutical compositions, e.g., as described in Remington's Pharmaceutical Sciences, 1985. For ease of administration, the monoclonal antibody will typically be combined with a pharmaceutically acceptable carrier. Such carriers include water, physiological saline, or oils.

Formulations suitable for parenteral administration include aqueous and non-
30 aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and

thickening agents. Except insofar as any conventional medium is incompatible with the active ingredient and its intended use, its use in any compositions is contemplated.

The formulations may be presented in unit- dose or multi-dose containers, for example, sealed ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use.

The OXYTOCIN RECEPTOR Antagonist nucleic acid molecules, polypeptides, and antibodies can be incorporated into pharmaceutical compositions suitable for administration. Such compositions typically comprise the nucleic acid molecule, protein, or antibody and a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

In another aspect, the invention relates to OXYTOCIN RECEPTOR Antagonists, as described herein, which are modified by the covalent attachment of a moiety. Such modification can produce an OXYTOCIN RECEPTOR Antagonist with improved pharmacokinetic properties (e.g., increased *in vivo* serum half-life). The organic moiety can be a linear or branched hydrophilic polymeric group, fatty acid group, or fatty acid ester group. In particular embodiments, the hydrophilic polymeric group can have a molecular weight of about 800 to about 120,000 Daltons and can be a polyalkane glycol (e.g., polyethylene glycol (PEG), polypropylene glycol (PPG)), carbohydrate polymer, amino acid polymer or polyvinyl pyrolidone, and the fatty acid or fatty acid ester group can comprise from about eight to about forty carbon atoms. As used herein, the term "fatty acid" encompasses mono-carboxylic acids and di-carboxylic acids. Fatty acids and fatty acid esters suitable for modifying antibodies of the invention can be saturated or can contain one or more units of unsaturation. Fatty acids that are suitable for modifying antibodies of the invention include, for example, n-dodecanoate (C_{12}, laurate), n-tetradecanoate (C_{14}, myristate), n-octadecanoate (C_{18}, stearate), n-eicosanoate (C_{20}, arachidate), n-docosanoate (C_{22}, behenate), n-triacontanoate (C_{30}), n-tetracontanoate (C_{40}), *cis*- delta 9-octadecanoate (C_{18}, oleate), all *cis*-delta5,8,11,14-eicosatetraenoate (C_{20}, arachidonate), octanedioic acid, tetradecanedioic acid, octadecanedioic acid, docosanedioic

acid, and the like. Suitable fatty acid esters include mono-esters of dicarboxylic acids that comprise a linear or branched lower alkyl group. The lower alkyl group can comprise from one to about twelve, preferably, one to about six, carbon atoms.

The modified human polypeptides and antibodies can be prepared using suitable methods, such as by reaction with one or more modifying agents. A "modifying agent" as the term is used herein, refers to a suitable organic group (e.g., hydrophilic polymer, a fatty acid, a fatty acid ester) that comprises an activating group. An "activating group" is a chemical moiety or functional group that can, under appropriate conditions, react with a second chemical group thereby forming a covalent bond between the modifying agent and the second chemical group. For example, amine-reactive activating groups include electrophilic groups such as tosylate, mesylate, halo (chloro, bromo, fluoro, iodo), N-hydroxysuccinimidyl esters (NHS), and the like. Activating groups that can react with thiols include, for example, maleimide, iodoacetyl, acrylolyl, pyridyl disulfides, 5-thiol-2-nitrobenzoic acid thiol (TNB-thiol), and the like. An aldehyde functional group can be coupled to amine- or hydrazide-containing molecules, and an azide group can react with a trivalent phosphorous group to form phosphoramidate or phosphorimide linkages. Suitable methods to introduce activating groups into molecules are known in the art (see for example, Hermanson, G. T., *Bioconjugate Techniques*, Academic Press: San Diego, CA (1996)).

The invention includes methods for preparing pharmaceutical compositions for modulating the expression or activity of an Oxytocin Receptor polypeptide, nucleic acid, or antibody. Such methods comprise formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of an Oxytocin Receptor polypeptide, nucleic acid, or antibody. Such compositions can further include additional active agents. Thus, the invention further includes methods for preparing a pharmaceutical composition by formulating a pharmaceutically acceptable carrier with an agent that modulates expression or activity of an Oxytocin Receptor polypeptide, nucleic acid, or antibody and one or more additional active compounds.

The agent that modulates expression or activity can, for example, be a small molecule. For example, such small molecules include peptides, peptidomimetics, amino acids, amino acid analogs, polynucleotides, polynucleotide analogs, nucleotides, nucleotide analogs, organic or inorganic compounds (i.e., including heteroorganic and organometallic compounds) having a molecular weight less than about 10,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 5,000 grams per mole, organic or inorganic compounds having a molecular weight less than about 1,000 grams per mole, organic

or inorganic compounds having a molecular weight less than about 500 grams per mole, and salts, esters, and other pharmaceutically acceptable forms of such compounds.

It is understood that appropriate doses of small molecule agents and protein or polypeptide agents depend upon a number of factors within the knowledge of the ordinarily skilled physician, veterinarian, or researcher. The dose(s) of these agents will vary, for example, depending upon the identity, size, and condition of the subject or sample being treated, further depending upon the route by which the composition is to be administered, if applicable, and the effect which the practitioner desires the agent to have upon the Oxytocin Receptor polypeptide, nucleic acid, or antibody. Exemplary doses of a small molecule include milligram or microgram amounts per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 500 milligrams per kilogram, about 100 micrograms per kilogram to about 5 milligrams per kilogram, or about 1 microgram per kilogram to about 50 micrograms per kilogram). Exemplary doses of a protein or polypeptide include gram, milligram or microgram amounts per kilogram of subject or sample weight (e.g., about 1 microgram per kilogram to about 5 grams per kilogram, about 100 micrograms per kilogram to about 500 milligrams per kilogram, or about 1 milligram per kilogram to about 50 milligrams per kilogram). It is furthermore understood that appropriate doses of one of these agents depend upon the potency of the agent with respect to the expression or activity to be modulated. Such appropriate doses can be determined using the assays described herein.

When one or more of these agents is to be administered to an animal (e.g., a human) in order to modulate expression or activity of an Oxytocin Receptor polypeptide, nucleic acid, or antibody, a physician, veterinarian, or researcher can, for example, prescribe a relatively low dose at first, subsequently increasing the dose until an appropriate response is obtained. In addition, it is understood that the specific dose level for any particular animal subject will depend upon a variety of factors including the activity of the specific agent employed, the age, body weight, general health, gender, and diet of the subject, the time of administration, the route of administration, the rate of excretion, any drug combination, and the degree of expression or activity to be modulated.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation or buccal), transdermal (topical), transmucosal, and rectal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluen, such as water for injection, saline solution, fixed oils, polyethylene glycols,

glycerine, propylene glycol or other synthetic solvents; antibacterial agents, such as benzyl alcohol or methyl parabens; antioxidants, such as ascorbic acid or sodium bisulfite; chelating agents, such as ethylenediamine-tetraacetic acid; buffers, such as acetates, citrates or phosphates and agents for the adjustment of tonicity, such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL (BASF; Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin. Pharmaceutical excipients and additives useful in stabilizing the present composition include, but are not limited to, polypeptides, peptides, amino acids, lipids, and carbohydrates (e.g., sugars, including monosaccharides, di-, tri-, tetra-, and oligosaccharides; derivatized sugars such as alditols, aldonic acids, esterified sugars and the like; and polysaccharides or sugar polymers), which can be present singly or in combination, comprising alone or in combination 1-99.99% by weight or volume. Exemplary but non-limiting polypeptide excipients include serum albumin, such as human serum albumin (HSA), recombinant human albumin (rHA), gelatin, casein, and the like. Representative amino acids, which can also function in a buffering capacity, include alanine, glycine, arginine, betaine, histidine, glutamic acid, aspartic acid, cysteine, lysine, leucine, isoleucine, valine, methionine, phenylalanine, aspartame, and the like. One preferred amino acid is glycine.

Sterile injectable solutions can be prepared by incorporating the active compound (e.g., a polypeptide or antibody) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium, and then incorporating the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Non-limiting examples of, and methods of preparing such sterile solutions are well known in the art, such as, but limited to, Gennaro, Ed., *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Co. (Easton, PA) 1990.

Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed.

Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches, and the like can contain any of the following ingredients, or compounds of a similar nature: a binder, such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient, such as starch or lactose, a disintegrating agent, such as alginic acid, Primogel, or corn starch; a lubricant, such as magnesium stearate or Sterotes; a glidant, such as colloidal silicon dioxide; a sweetening agent, such as sucrose or saccharin; or a flavoring agent, such as peppermint, methyl salicylate, or orange flavoring.

For administration by inhalation, the compounds are delivered in the form of aerosolized particles from a pressurized container or dispenser that contains a suitable propellant, e.g., a gas such as carbon dioxide, or a nebulizer. Alternatively, compositions formulated as particles can be dispersed by electrostatic, mechanical means including vibrations, or ultrasonic means as taught in U.S. Patent Nos: 4530464; 4533082; 5838350; 6113001; 6514496; 5518179; 5152456; 5261601; and 4605167.

Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be

permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams, as generally known in the art.

The compounds can also be prepared in the form of suppositories (e.g., with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

In one embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions (including liposomes having monoclonal antibodies incorporated therein or thereon) can also be used as pharmaceutically acceptable carriers. Particularly preferred compositions and methods are taught in U.S. Pat. Nos. 5,891,468 and 6,316,024.

It is especially advantageous to formulate oral or parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of individuals.

For antibodies, the preferred dosage is about 0.1 mg/kg to 100 mg/kg of body weight (generally about 10 mg/kg to 20 mg/kg). If the antibody is to act in the brain, a dosage of about 50 mg/kg to 100 mg/kg is usually appropriate. Generally, partially human antibodies and fully human antibodies have a longer half-life within the human body than other antibodies. Accordingly, the use of lower dosages and less frequent administration is often possible. Modifications, such as lipidation, can be used to stabilize antibodies and to enhance uptake and tissue penetration (e.g., into the brain). A method for lipidation of antibodies is described by

Cruikshank et al. ((1997) *J. Acquired Immune Deficiency Syndromes and Human Retrovirology* 14:193).

The OXYTOCIN RECEPTOR Antagonist nucleic acid molecules can be inserted into vectors and used as gene therapy vectors. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (U.S. Pat. No. 5,328,470), or by stereotactic injection (see, e.g., Chen et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:3054- 3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g. retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

Pharmacogenomics

Agents, or modulators that have a stimulatory or inhibitory effect on activity or expression of an Oxytocin Receptor polypeptide as identified by a screening assay described herein, can be administered to individuals to treat (prophylactically or therapeutically) disorders associated with aberrant activity of the polypeptide. In conjunction with such treatment, the pharmacogenomics (i.e., the study of the relationship between an individual's genotype and that individual's response to a foreign compound or drug) of the individual may be considered. Differences in metabolism of therapeutics can lead to severe toxicity or therapeutic failure by altering the relation between dose and blood concentration of the pharmacologically active drug. Thus, the pharmacogenomics of the individual permits the selection of effective agents (e.g., drugs) for prophylactic or therapeutic treatments based on a consideration of the individual's genotype. Such pharmacogenomics can further be used to determine appropriate dosages and therapeutic regimens. Accordingly, the activity of an Oxytocin Receptor polypeptide, expression of an Oxytocin Receptor nucleic acid, or mutation content of an Oxytocin Receptor gene in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual.

Pharmacogenomics deals with clinically significant hereditary variations in the response to drugs due to altered drug disposition and abnormal action in affected persons. See, e.g., Linder (1997) *Clin. Chem.* 43(2):254-266. In general, two types of pharmacogenetic conditions can be differentiated. Genetic conditions transmitted as a single factor altering the

way drugs act on the body are referred to as "altered drug action." Genetic conditions transmitted as single factors altering the way the body acts on drugs are referred to as "altered drug metabolism." These pharmacogenetic conditions can occur either as rare defects or as polymorphisms. For example, glucose-6-phosphate dehydrogenase (G6PD) deficiency is a common inherited enzymopathy in which the main clinical complication is hemolysis after ingestion of oxidant drugs (anti-malarials, sulfonamides, analgesics, nitrofurans) and consumption of fava beans.

As an illustrative embodiment, the activity of drug metabolizing enzymes is a major determinant of both the intensity and duration of drug action. The discovery of genetic polymorphisms of drug metabolizing enzymes (e.g., N-acetyltransferase 2 (NAT 2) and cytochrome P450 enzymes CYP2D6 and CYP2C19) has provided an explanation as to why some patients do not obtain the expected drug effects or show exaggerated drug response and serious toxicity after taking the standard and safe dose of a drug. These polymorphisms are expressed in two phenotypes in the population, the extensive metabolizer (EM) and poor metabolizer (PM). The prevalence of PM is different among different populations. For example, the gene coding for CYP2D6 is highly polymorphic and several mutations have been identified in PM, which all lead to the absence of functional CYP2D6. Poor metabolizers of CYP2D6 and CYP2C19 quite frequently experience exaggerated drug response and side effects when they receive standard doses. If a metabolite is the active therapeutic moiety, a PM will show no therapeutic response, as demonstrated for the analgesic effect of codeine mediated by its CYP2D6-formed metabolite morphine. The other extreme are the so called ultra-rapid metabolizers who do not respond to standard doses. Recently, the molecular basis of ultra-rapid metabolism has been identified to be due to CYP2D6 gene amplification.

Thus, the activity of an Oxytocin Receptor polypeptide, expression of a nucleic acid encoding the polypeptide, or mutation content of a gene encoding the polypeptide in an individual can be determined to thereby select appropriate agent(s) for therapeutic or prophylactic treatment of the individual. In addition, pharmacogenetic studies can be used to apply genotyping of polymorphic alleles encoding drug-metabolizing enzymes to the identification of an individual's drug responsiveness phenotype. This knowledge, when applied to dosing or drug selection, can avoid adverse reactions or therapeutic failure and thus enhance therapeutic or prophylactic efficiency when treating a subject with a modulator of activity or expression of the polypeptide, such as a modulator identified by one of the exemplary screening assays described herein.

Methods of Treatment

The present invention provides for both prophylactic and therapeutic methods of treating a subject at risk of (or susceptible to) a disorder or having a disorder associated with aberrant expression or activity of an Oxytocin Receptor polypeptide and/or in which the Oxytocin Receptor polypeptide is involved.

The present invention provides a method for modulating or treating at least one Oxytocin Receptor related disease or condition, in a cell, tissue, organ, animal, or patient, as known in the art or as described herein, using at least one OXYTOCIN RECEPTOR Antagonist.

Compositions of OXYTOCIN RECEPTOR Antagonists may find therapeutic use in the treatment of pulmonary disorder-related conditions, such as asthma, emphysema, COPD, and neonatal chronic lung disease.

The present invention also provides a method for modulating or treating at least one immune related disease, in a cell, tissue, organ, animal, or patient including, but not limited to, at least one of rheumatoid arthritis, juvenile rheumatoid arthritis, systemic onset juvenile rheumatoid arthritis, psoriatic arthritis, ankylosing spondilitis, gastric ulcer, seronegative arthropathies, osteoarthritis, inflammatory bowel disease, ulcerative colitis, systemic lupus erythematosis, antiphospholipid syndrome, iridocyclitis/uveitis/optic neuritis, idiopathic pulmonary fibrosis, systemic vasculitis/wegener's granulomatosis, sarcoidosis, orchitis/vasectomy reversal procedures, allergic/atopic diseases, allergic rhinitis, eczema, allergic contact dermatitis, allergic conjunctivitis, hypersensitivity pneumonitis, transplants, organ transplant rejection, graft-versus-host disease, systemic inflammatory response syndrome, sepsis syndrome, gram positive sepsis, gram negative sepsis, culture negative sepsis, fungal sepsis, neutropenic fever, urosepsis, meningococcemia, trauma/hemorrhage, burns, ionizing radiation exposure, acute pancreatitis, adult respiratory distress syndrome, rheumatoid arthritis, alcohol-induced hepatitis, chronic inflammatory pathologies, sarcoidosis, Crohn's pathology, sickle cell anemia, diabetes, nephrosis, atopic diseases, hypersensitity reactions, allergic rhinitis, hay fever, perennial rhinitis, conjunctivitis, endometriosis, urticaria, systemic anaphalaxis, dermatitis, pernicious anemia, hemolytic disease, thrombocytopenia, graft rejection of any organ or tissue, kidney translplant rejection, heart transplant rejection, liver transplant rejection, pancreas transplant rejection, lung transplant rejection, bone marrow transplant (BMT) rejection, skin allograft rejection, cartilage transplant rejection, bone graft rejection, small bowel transplant rejection, fetal thymus implant rejection, parathyroid transplant rejection, xenograft rejection of any organ or tissue, allograft rejection, anti-receptor hypersensitivity reactions, Graves disease, Raynoud's disease, type B insulin-resistant

diabetes, myasthenia gravis, antibody-meditated cytotoxicity, type III hypersensitivity reactions, systemic lupus erythematosus, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), antiphospholipid syndrome, pemphigus, scleroderma, mixed connective tissue disease, idiopathic Addison's disease, diabetes mellitus, chronic active hepatitis, primary billiary cirrhosis, vitiligo, vasculitis, post-MI cardiotomy syndrome, type IV hypersensitivity, contact dermatitis, hypersensitivity pneumonitis, allograft rejection, granulomas due to intracellular organisms, drug sensitivity, metabolic/idiopathic, Wilson's disease, hemachromatosis, alpha-1-antitrypsin deficiency, diabetic retinopathy, hashimoto's thyroiditis, osteoporosis, hypothalamic-pituitary-adrenal axis evaluation, primary biliary cirrhosis, thyroiditis, encephalomyelitis, cachexia, cystic fibrosis, familial hematophagocytic lymphohistiocytosis, dermatologic conditions, psoriasis, alopecia, nephrotic syndrome, nephritis, glomerular nephritis, acute renal failure, hemodialysis, uremia, toxicity, preeclampsia, okt3 therapy, anti-cd3 therapy, cytokine therapy, chemotherapy, radiation therapy (e.g., including but not limited toasthenia, anemia, cachexia, and the like), chronic salicylate intoxication, and the like. See, e.g., the Merck Manual, 12th-17th Editions, Merck & Company, Rahway, NJ (1972, 1977, 1982, 1987, 1992, 1999), Pharmacotherapy Handbook, Wells et al., eds., Second Edition, Appleton and Lange, Stamford, Conn. (1998, 2000), each entirely incorporated by reference.

The present invention also provides a method for modulating or treating at least one malignant disease in a cell, tissue, organ, animal or patient, including, but not limited to, at least one of: leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), acute promyelocytic leukemia (APL), chromic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), hairy cell leukemia, myelodyplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignamt lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, Kaposi's sarcoma, colorectal carcinoma, pancreatic carcinoma, nasopharyngeal carcinoma, malignant histiocytosis, paraneoplastic syndrome/hypercalcemia of malignancy, solid tumors, adenocarcinomas, sarcomas, malignant melanoma, hemangioma, metastatic disease, cancer related bone resorption, cancer related bone pain, and the like.

Disorders characterized by aberrant expression or activity of the Oxytocin Receptor polypeptides are further described elsewhere in this disclosure.

1. Prophylactic Methods

In one aspect, the invention provides a method for at least substantially preventing in a subject, a disease or condition associated with an aberrant expression or activity of an Oxytocin Receptor polypeptide, by administering to the subject an agent that modulates expression or at least one activity of the polypeptide. Subjects at risk for a disease that is caused or contributed to by aberrant expression or activity of an Oxytocin Receptor polypeptide can be identified by, for example, any or a combination of diagnostic or prognostic assays as described herein. Administration of a prophylactic agent can occur prior to the manifestation of symptoms characteristic of the aberrancy, such that a disease or disorder is prevented or, alternatively, delayed in its progression. Depending on the type of aberrancy, for example, an agonist or antagonist agent can be used for treating the subject. The appropriate agent can be determined based on screening assays described herein.

2. Therapeutic Methods

Another aspect of the invention pertains to methods of modulating expression or activity of an Oxytocin Receptor polypeptide for therapeutic purposes. The modulatory method of the invention involves contacting a cell with an agent that modulates one or more of the activities of the polypeptide. An agent that modulates activity can be an agent as described herein, such as a nucleic acid or a protein, a naturally-occurring cognate ligand of the polypeptide, a peptide, a peptidomimetic, or other small molecule. In one embodiment, the agent stimulates one or more of the biological activities of the polypeptide. In another embodiment, the agent inhibits one or more of the biological activities of the Oxytocin Receptor polypeptide. Examples of such inhibitory agents include antisense nucleic acid molecules and antibodies and other methods described herein. These modulatory methods can be performed in vitro (e.g., by culturing the cell with the agent) or, alternatively, in vivo (e.g., by administering the agent to a subject). As such, the present invention provides methods of treating an individual afflicted with a disease or disorder characterized by aberrant expression or activity of an Oxytocin Receptor polypeptide. In one embodiment, the method involves administering an agent (e.g., an agent identified by a screening assay described herein), or combination of agents that modulate (e.g., up-regulates or down-regulates) expression or activity. Inhibition of activity is desirable in situations in which activity or expression is abnormally high or up-regulated and/or in which decreased activity is likely to have a beneficial effect.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples which should not be construed as limiting the scope of the claims.

EXAMPLE 1

5 Microarray Experiment Description

Total RNA was collected from treated primary cultures of human airway smooth muscle cells (HASMC). The quiesced control HASMC were grown for 4 weeks (in 6 passages in order to allow for expansion of cell number so that enough RNA could be isolated) in 10% FBS/DMEM, quiesced in 1% FBS/DMEM for 24 hours and in 0% FBS/DMEM for an additional 10 24 hours. HASMC were exposed to atopic sera (from individuals that were hyper-sensitive to an antigen or allergen) or non-atopic sera for up to 24 hours. As used herein, the terms "atopy" and "atopic" are defined as a genetic predisposition to become IgE sensitized to allergens commonly occurring in the environment and to which everyone is exposed but to which the majority do not produce a prolonged IgE antibody response. Microarray analysis was 15 conducted on RNA from asthmatic and non-asthmatic HASMC that were left unstimulated or stimulated with atopic or non-atopic serum for 15 minutes, 30 minutes, 2 hours, 4 hours, 8 hours or 24 hours.

Chip Type: An internally developed cDNA microarray TargetA_1, TargetA_2 is used.

20 **Data Analysis**

There were 28 possible combinations of the experimental conditions. These conditions are in the following categories (1) the disease state: asthma or non-asthma; (2) treatment: atopic or non-atopic serum; and (3) time point: 0, 15 minutes, 30 minutes, 2 hours, 4 hours, 8 hours or 24 hours.

25 Replicate samples were grouped according to their experimental conditions. The average of normalized intensities was used to represent each condition.

Analysis I

Without the assumption of equal variances, parametric analysis using Welch's approximate t-test was done for the following comparisons (from categories (1) and (2) above) 30 at each corresponding time point (category (3)):

A. Asthmatic non-atopic serum vs. nonasthmatic non-atopic serum
B. Asthmatic atopic serum vs. asthmatic non-atopic serum
C. Asthmatic atopic serum vs. non-asthmatic atopic serum
D. Non-asthmatic atopic serum vs. non-asthmatic non-atopic serum

At each of the above 4 categories, common genes showing significant (P-value < 0.05) differential RNA expression in the same direction at multiple time points of the time course were identified.

The fold change of differential RNA expression for each comparison was calculated.

The genes being identified multiple times in comparisons A and C are presumed to be the genes related to asthmatic conditions. The genes being identified multiple times in comparisons B and D should be the genes related to atopy.

Analysis II

This analysis was to identify genes showing up-regulation in one of the asthmatic conditions regardless of at which time point the peak appeared. The similar differential expression was not observed in any of the non-asthmatic conditions. The following four conditions were considered in this analysis:

E. A_atop (asthmatic/atopic),
F. A_noatop (asthmatic/non-atopic),
G. N_atop (non-asthmatic/atopic), and
H. N_noatop (non-asthmatic/non-atopic)

The procedure used to make the identification was as follows:

Within an asthmatic condition, pair wise comparisons were done for each time point vs. A_cntl and the other time points. The identified genes show higher RNA expression than at any of the other time points, and greater than a 2-fold RNA expression change as compared to the baseline, A_cntl.

A similar identification was done for each time point of the same condition. The gene lists were then combined for the condition, containing the genes showing RNA expression peaks on at least one time point.

These two steps were repeated for the other three conditions. The genes being identified for an asthmatic condition, excluding the ones being identified in a similar manner for the other three conditions, were up regulated specifically in this asthmatic condition.

Analysis III:

The RNA expression patterns of more than 40 genes that are known from scientific literature to be asthma-related were plotted in the experiment. Many of them show RNA expression profiles matching the published data.

Analysis IV:

The expression profiles of the genes identified in analysis I, II, or III were used as reference profiles to search for genes associated with similar RNA expression profiles. Each

gene (RNA) expression profile must have the defined minimum correlation, 0.95 to 0.99, to be considered similar. The higher the minimum correlation (maximum 1.0), the closer the gene (RNA) expression profiles would be.

Annotations for identified genes were obtained from GenBank, LocusLink,
5 Unigene, and Gene Ontology.

Microarray results for Oxytocin Receptor gene

From the above analyses of the microarray data, as shown in Fig. 1, the Oxytocin Receptor displayed an elevated RNA expression (1.5 fold up) in the asthmatic cells with atopic serum treatment compared to non-asthmatic cells with atopic serum treatment.
10 Similarly, as shown in Fig. 2, there was an elevated RNA expression (1.4 fold up) in asthmatic cells with non-atopic serum treatment compared to non-asthmatic cells with non-atopic serum treatment.

Real Time PCR analysis of airway smooth muscle cells

In order to confirm the microarray finding by an independent means, Real Time
15 PCR technology was employed. Primary human airway smooth muscle cells from "normal" donors were cultured with IL-13 or a naturally occurring mutant form IL-13R130Q (associated with high serum IgE levels) for 0, 6 hours or 18 hours at 50 ng/ml after allowing the cell to rest for 24 hours. Total RNA was isolated, reverse transcribed and used in Real Time PCR analysis for oxytocin receptor using the Applied Biosystems Gene Expression Assay (ID number
20 Hs00168573_m1) on the ABI PRISM® 7900HT Sequence Detection System. At 6 hours, the level of OXTR mRNA normalized to 18S mRNA and relative to untreated control (i.e., 0 hour time point) was 1.95 (Std Dev. 0.41) when cells were stimulated with WT IL-13 and 1.79 (Std Dev. 0.22) when stimulated with R130Q. At 18 hours, the level of OXTR mRNA normalized to 18S mRNA and relative to untreated control (i.e., 0 hour timepoint) was 2.02 (Std Dev. 0.42)
25 when the cells were stimulated with WT IL-13 and 3.58 (Std Dev. 0.74) when stimulated with R130Q.

The results demonstrated that the Oxytocin Receptor was expressed in primary airway smooth muscle cells and the expression was further induced by IL-13 up to 2-fold, and by IL-13R130Q up to 2-fold at 6 hours and approximately 3.5-fold at 18 hours.

30 Primary human airway smooth muscle cells from two "normal" donors were cultured with tumor necrosis factor alpha (TNFα) for 0, 6 hours or 18 hours at 10 ng/ml after resting the cells for 24 hours. Total RNA was isolated, reverse transcribed and used in Real Time PCR analysis for the Oxytocin Receptor. TNFα induced the expression of the Oxytocin

Receptor by 4.88 (Std Dev. 1.13) at 6 hours and 7.06 (Std. Dev. 1.42) at the 18-hour time point normalized to 18S and relative to untreated control (i.e., 0 hour timepoint).

These data confirmed the novel RNA expression of Oxytocin Receptor on human airway smooth muscle cells and the expression was regulated by inflammatory cytokines, which supports the potential role of Oxytocin Receptor in the pathogenesis of pulmonary inflammatory diseases.

Real Time PCR analysis of an array of human tissues

In order to evaluate the RNA expression of the Oxytocin Receptor across various tissues, Real Time PCR was employed using the Applied Biosystems Gene Expression Assay (ID number Hs00168573_m1) on the ABI PRISM® 7900HT Sequence Detection System to detect the Oxytocin Receptor in various normal tissues. The Oxytocin Receptor levels (designated "18S adj Qty") in various tissues are shown in Table 1.

The highest expression was detected in breast tissue, followed by tissues of the male caval vein and penis and uterus. The RNA expression level was fairly low for the majority of normal tissues, including normal lung. This suggests that the expression of *OXTR* in lung for instance probably is up regulated in a diseased or an inflamed condition. In Table 1, the tissue type abbreviations are as follows: RP = Reproductive; CV = Cardiovascular; GI = Gastrointestinal; S = Soft tissue/Organ; GU = Genitourinary; and R = Respiratory.

Table 1

Tissue Type	Sex	Sample for Total RNA Human Adult	18S adj Qty
RP	F	Breast, Female, Adult	160.23
RP	M	Caval Vein, Male, Adult	25.39
RP	M	Penis, Male, Adult	25.36
RP	F	Uterus, Female, Adult	13.79
GI	F	Colon, Ascending, Female, Adult	12.49
CV	F	Heart, Female, Fetal	12.02

S	M	Brain, Occipital Cortex, Male, Adult	11.65
RP	F	Ovary, Female, Adult	11.60
S	F	Brain, Female, Fetal	10.36
S	M	Brain, Male, Adult	9.02
RP	M	Testes, Male, Adult	8.61
S	M	Brain, Parietal Cortex, Male, Adult	8.35
R	M	Lung, Male, Fetal	8.34
S	M, F	Spleen, Female/Male pooled, Fetal	7.77
GU	M	Bladder, Male, Fetal	7.29
CV	M	Lymph Node, Male, Adult	6.65
GI	F	Stomach, Female, Fetal	6.28
RP	F	Placenta, Adult, Female	6.04
R	M	Larynx, Male, Adult	5.88
S	F	Kidney, Female, Fetal	5.79
R	F	Trachea, Female, Adult	5.56
R	M	Larynx, Male, Adult (Normal)	5.56
GU	F	Bladder, Female, Fetal	5.45
S	M	Kidney, Male, Fetal	4.41
RP	M	Prostate, Male, Adult	4.24

GI	M	Stomach, Male, Fetal	3.92
R	M	Lung, Male, Adult	3.58
GU	M	Bladder, Male, Adult	3.46
R	M	Larynx, Male, Adult	3.36
GI	M	Colon, Male, Adult	3.21
S	F	Kidney, Female, Adult	3.18
CV	F	Aorta, Female, Fetal	3.13
GI	F	Colon, Female, Adult (Top)	3.05
CV	M	Pericardium, Male, Adult	2.79
S	M	Kidney, Male, Adult	2.72
GI	F	Colon, Female, Fetal	2.67
S	M	Brain, Male, Fetal	2.66
S	F	Skin, Female, Fetal	2.65
S	M	Skeletal Muscle, Male, Fetal	2.49
S	M	Spleen, Male, Adult	2.48
S	M	Liver, Male, Fetal	2.04
R	F	Lung, Female, Adult	1.97
GI	M	Colon, Male, Fetal	1.92
S	M, F	Tongue, Male/Female, Adult	1.90
S	F	Skeletal Muscle, Female, Fetal	1.90
R	F	Lung, Female, Fetal	1.88
S	F	Spleen, Female, Adult	1.86

RP	F	Cervix, Female, Adult	1.78
S	M	Thymus, Male, Adult	1.78
S	M, F	Thymus, Male and Female, Fetal	1.64
S	F	Liver, Female, Fetal	1.40
S	F	Thyroid, Female, Adult	1.34
GI	M	Colon, Male, Adult (Normal)	1.30
S	M	Skin, Male, Adult	1.05
GI	F	Colon, Decending, Female, Adult	1.03
S	F	Adrenal, Female, Adult	1.00
S	F	Skin, Female, Adult	0.93
CV	M	Heart, Left Atrium, Male, Adult	0.86
GI	M	Rectum, Male, Adult	0.79
S	M	Liver, Male, Adult	0.62
GI	M	Stomach, Male, Adult	0.61
S	F	Liver, Female, Adult	0.44
GI	F	Parotid, Female, Adult	0.41
CV	M	Heart, Male, Adult	0.34
S	M	Pancreas, Male, Adult	0.28
S	M	Skeletal Muscle, Male, Adult	0.23
GI	F	Stomach, Female, Adult	0.19
CV	F	Heart, Female, Adult	0.16

Protein Expression

To detect the protein levels of the Oxytocin Receptor in human airway smooth muscle cells, total cell lysate from primary human airway smooth muscle cells was electrophoresed and immunoblotted with an anti-OXYTOCIN RECEPTOR antibody. Fig. 3 shows the expression of Oxytocin Receptor in human airway smooth muscle cells (HASMC) along with the positive control (MDAMB231).

Oxytocin receptor was clearly detected in the cell lysates with similar molecular weight as that detected in a positive cell line. These results are the first demonstration that Oxytocin Receptor protein is expressed by human airway smooth muscle cells.

Functional data

In order to determine whether the Oxytocin Receptor expressed on the surface of human airway smooth muscle cells is functional, the amount of influx of calcium was measured. HASMC were serum starved and then either unstimulated or stimulated with IL-13 (50 ng/ml) for 24 hours. The amount of calcium that mobilized into the cells was measured after addition of oxytocin (100 nM or 500 nM). Fig. 4 shows that oxytocin induces Ca influx in human airway smooth muscle cells, and at low concentrations of oxytocin, IL-13 further enhances calcium influx in HASMC induced by oxytocin.

In vivo data

The RNA expression of Oxytocin Receptor in an *in vivo* model of acute asthma was tested. The mice were immunized with ovalbumin intraperitoneal on day 1 and day 8 and then challenged intranasally on days 22, 23 and 24. On day 25, the mice were euthanized. The trachea were extracted for RNA isolation and used in Real Time PCR analysis for Oxytocin Receptor.

Oxytocin Receptor mRNA was observed to be 1.5 fold higher in trachea from mice immunized and challenged with ovalbumin compared to PBS control.

Advantage/Utilities

In summary, a novel association for the Oxytocin Receptor gene/protein regulation with IL13 in human airway smooth muscle cells has been discovered. Oxytocin Receptor has classically been known to be involved in uterine contractions during parturition, milk ejection during lactation and male ejaculation. Herein, this discovery implicates oxytocin-Oxytocin Receptor binding in the airway smooth muscle, its reactivity, and its involvement in

pulmonary diseases are demonstrated. It can be hypothesized that the inflammatory cytokines, such as IL-13 and TNFα, in the inflamed lung lead to hyper-responsiveness to oxytocin induced smooth muscle contraction of the airways (due to an increased OXTR expression) leading to bronchoconstriction and hence a reduction in the air flow.

5 The level of Oxytocin Receptor mRNA was up-regulated by proinflammatory cytokines, such as IL-13 and TNFα, indicating in the asthmatic lung, airway smooth muscle cells have enhanced sensitivity to oxytocin and more prone to contraction during cytokine release and therefore may contribute to the symptomology of asthma attacks. Therefore, the Oxytocin Receptor is a biomarker for diagnosis of hypersensitized individuals as well as being a 10 target for the development of therapeutics useful in treating various immune-mediated inflammatory disorders, such as asthma.

As a target, the Oxytocin Receptor is amenable for antagonists thereto including, without limitation, monoclonal antibodies or small molecule compounds. Antibodies selective for the Oxytocin Receptor can be produced for diagnostic, prognostic, or therapeutic 15 purposes. Alternatively, OXYTOCIN RECEPTOR antagonists can be used in combination with current anti-cytokine therapies to better modulate the expression of various pro-inflammatory cytokines in different disease settings as mentioned. Such interventions may also be used, for example, for the prevention and treatment of pulmonary-related disorders or diseases such as asthma, COPD and emphysema. Targeted delivery to the lung via the intransal route would 20 provide the added benefit of avoiding undesirable side effects.

EXAMPLE 2

SiRNA and DNAzyme Antagonists

The role of Oxytocin Receptor in the progression of asthma or other respiratory system pathologies can be elucidated by modulating gene expression by the RNAi pathway or 25 by the use of specific DNAzymes. Similarly, the Oxytocin Receptor can be overexpressed by the use of vectors containing a strong promoter. Stable cell lines suppressed for Oxytocin Receptor expression and overexpressing Oxytocin Receptor can be generated. These cell lines can then be used in in vitro functional studies allowing the rapid validation of Oxytocin Receptor as a target for inflammatory disorders, such as the pulmonary-disorders described herein. To 30 this end, siRNAs specific for the Oxytocin Receptor are screened and then effective siRNAs are cloned into vectors as hairpins allowing the generation of stable cell lines. Additionally, Oxytocin Receptor-specific DNAzymes are designed and tested. Overexpression clones are also generated.

Although illustrated and described above with reference to certain specific embodiments, the present invention is nevertheless not intended to be limited to the details shown. Rather, the present invention is directed to the OXYTOCIN RECEPTOR antagonist polypeptides, polynucleotides, antibodies, apparatus, and kits disclosed herein and uses
5 thereof, and methods for controlling the levels of the Oxytocin Receptor, and various modifications may be made in the details within the scope and range of equivalents of the claims and without departing from the spirit of the invention.

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CLAIMS

We claim:

1. A method for treating an Oxytocin Receptor activity-related disorder in a cell, tissue, organ or animal comprising administering to the cell, tissue, organ or animal an OXYTOCIN RECEPTOR antagonist in an amount effective to inhibit the Oxytocin Receptor activity in said cell, tissue, organ or animal.
2. The method of claim 1 wherein the OXYTOCIN RECEPTOR antagonist is an OXYTOCIN RECEPTOR monoclonal antibody or a fragment thereof.
3. The method according to claim 2, wherein the antibody fragment is a Fab, Fab', or F(ab')2 fragment or derivative thereof.
4. The method according to claim 2 wherein the animal is a mammal.
5. The method according to claim 4, wherein the monoclonal antibody is administered by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, intralesional, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.
6. The method according to claim 5, wherein the monoclonal antibody is administered intravenously.
7. The method according to claim 5, wherein the monoclonal antibody is administered in the amount of from about 0.05 mg/kg to about 12.0 mg/kg body weight of said mammal.
8. The method according to claim 5, wherein the mammal is a human patient.
9. The method according to claim 2, wherein the monoclonal antibody is administered in a bolus dose followed by an infusion of said antibody.
10. The method according to claim 1, wherein the Oxytocin Receptor activity-related disorder is an inflammatory disorder.
11. The method according to claim 1, wherein the Oxytocin Receptor activity-related disorder is a pulmonary-related disorder.

12. The method according to claim 1, wherein the Oxytocin Receptor activity-related disorder is selected from the group consisting of asthma, emphysema, and chronic obstructive pulmonary disorder (COPD).

13. The method according to claim 1 wherein the OXYTOCIN RECEPTOR antagonist is selected from the group consisting of a small molecule, a polynucleotide, and a polypeptide.

14. The method according to claim 13 wherein the OXYTOCIN RECEPTOR antagonist is selected from the group consisting of an siRNA, shRNA, and DNAzyme molecule.

15. A method for diagnosing an Oxytocin Receptor activity-related disorder in a cell, tissue, organ or animal comprising administering to the cell, tissue, organ or animal an OXYTOCIN RECEPTOR antagonist in an amount effective to detect the Oxytocin Receptor activity in said cell, tissue, organ or animal.

16. Any invention described herein.

Figure 1

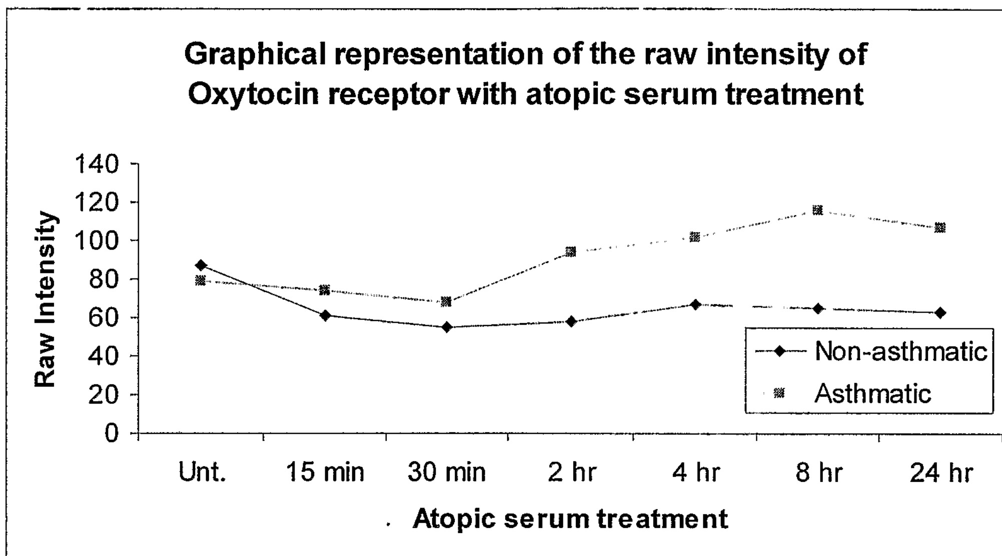


Figure 2

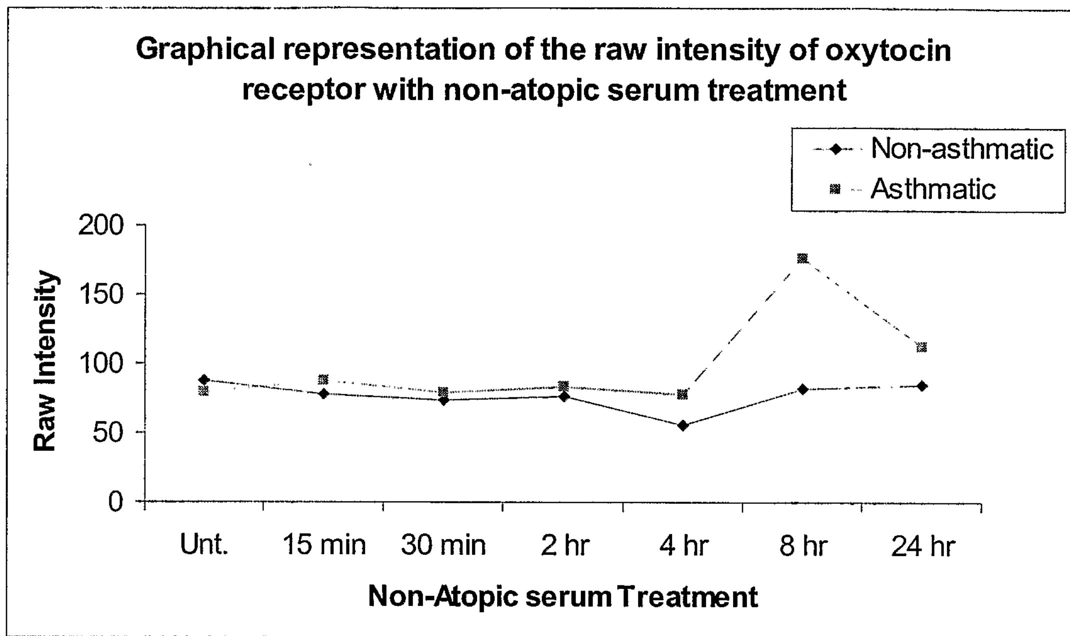


Figure 3

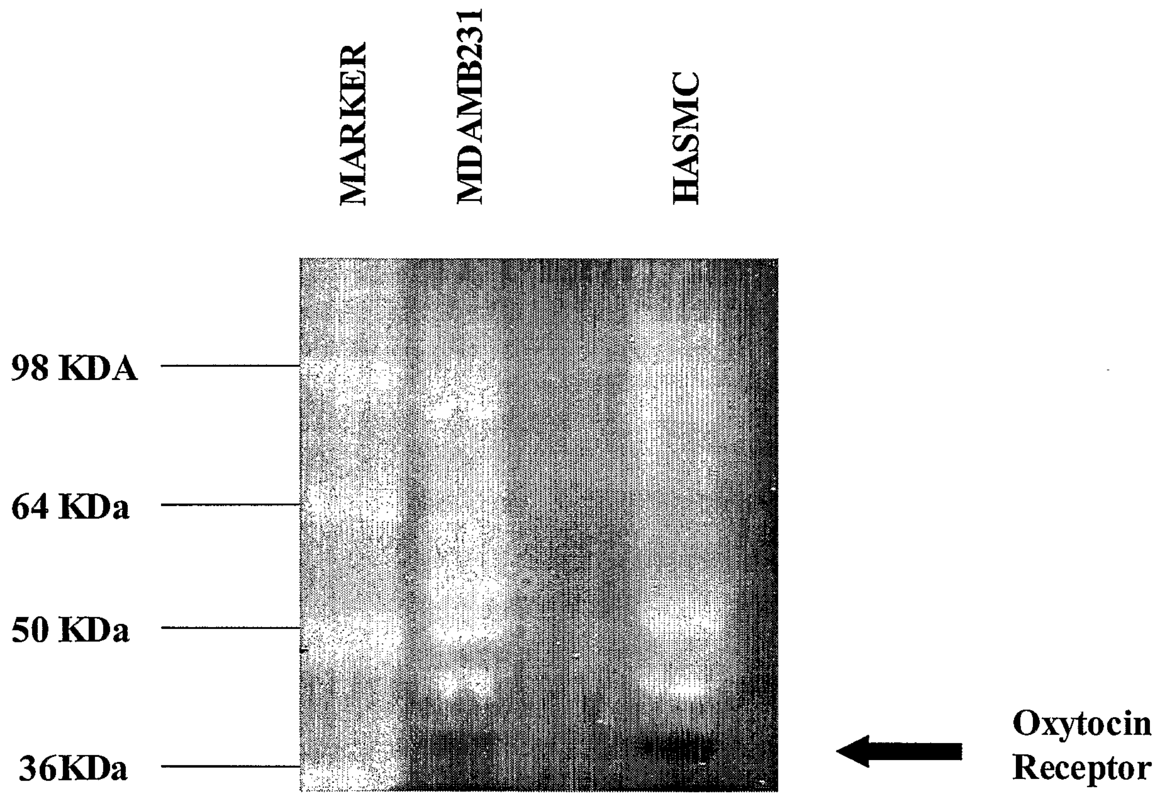
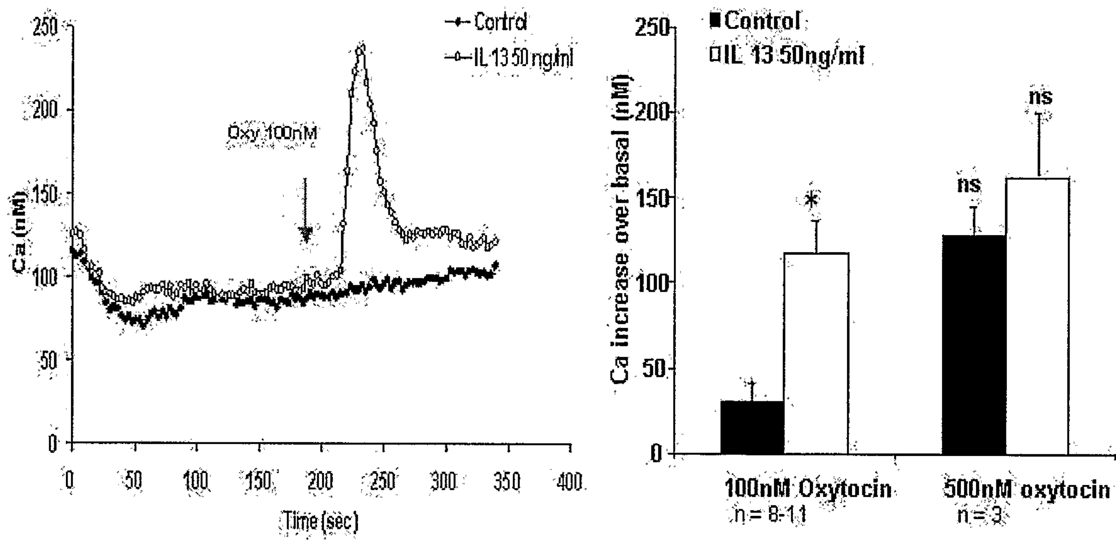


Figure 4

Oxytocin-mediated calcium release in human airway smooth muscle cells



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