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(54) ANTISENSE COMPOSITIONS TARGETED TO BETA1-ADRENOCEPTOR-SPECIFIC MRNA AND METHODS OF USE

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(57) **ABSTRACT**

Disclosed are antisense oligonucleotide, polynucleotide, and peptide nucleic acid compounds that specifically bind to mammalian mRNA encoding a β_1-adrenoceptor polypeptide and that are useful in the control and/or treatment of cardiac dysfunction, hypertension, hypertrophy, myocardial ischemia, and other cardiovascular diseases in an affected mammal, and preferably, in a human subject. The antisense compounds disclosed herein, and pharmaceutical formulations thereof, provide sustained control of β_1-adrenoceptor expression over prolonged periods, and achieve therapeutic effects from as little as a single dose. Administration of these antisense compositions to approved animal models resulted in a decrease in blood pressure, but no significant change in heart rate. Use of such antisense compositions in the reduction of β_1-adrenoceptor polypeptides in a host cell expressing β_1-adrenoceptor-specific mRNA, and in the preparation of medicaments for treating human and animal diseases, and in particular, hypertension and other cardiac dysfunction is also disclosed.

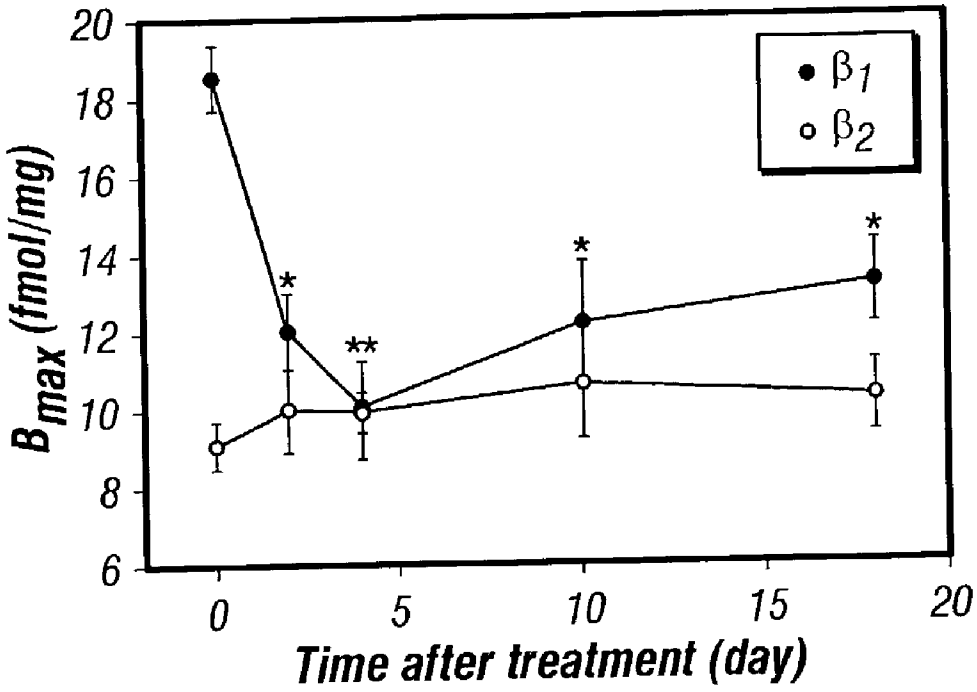
20
18
16
14
12
10
8
6
B_{max} (fmol/mg)
β_1
β_2
*
**
*
*
0
5
10
15
20
Time after treatment (day)

FIG. 1A

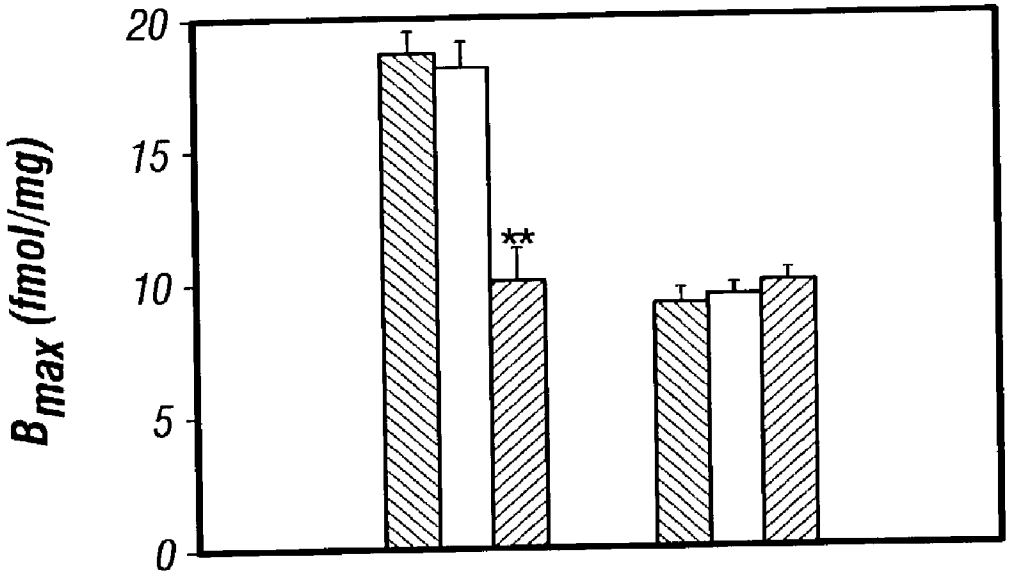
20
15
10
5
0
B_{max} (fmol/mg)
**

FIG. 1B

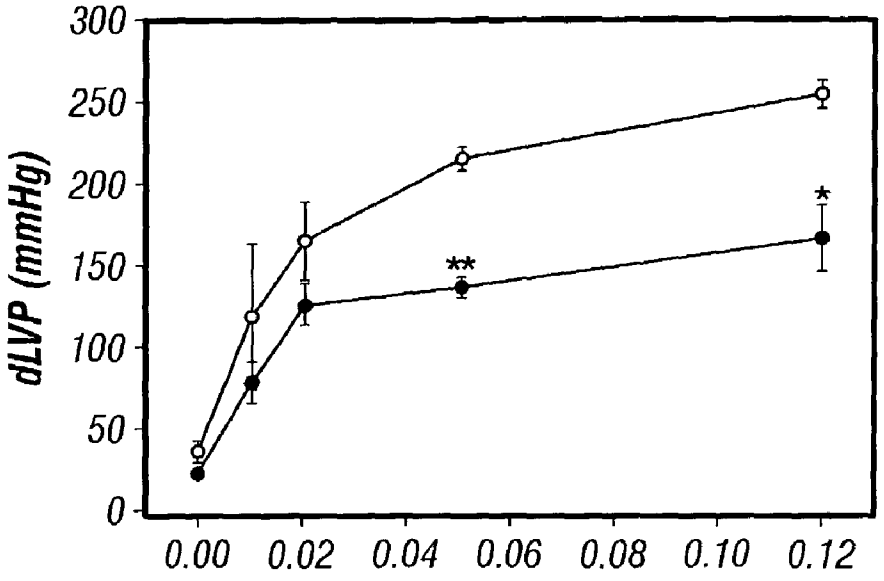
dLVP (mmHg)
300
250
200
150
100
50
0
0.00
0.02
0.04
0.06
0.08
0.10
0.12
**
*

FIG. 2A

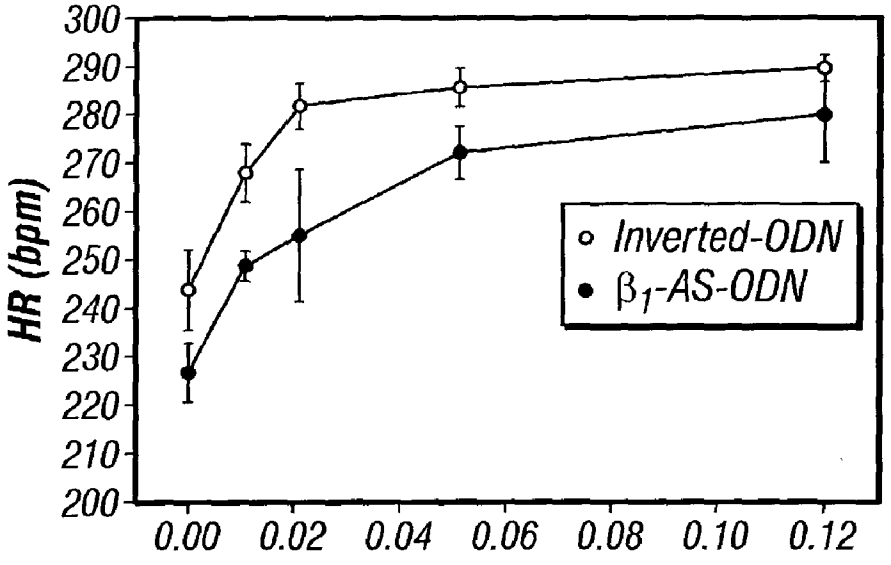
HR (bpm)
300
290
280
270
260
250
240
230
220
210
200
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Inverted-ODN
β1-AS-ODN

FIG. 2B

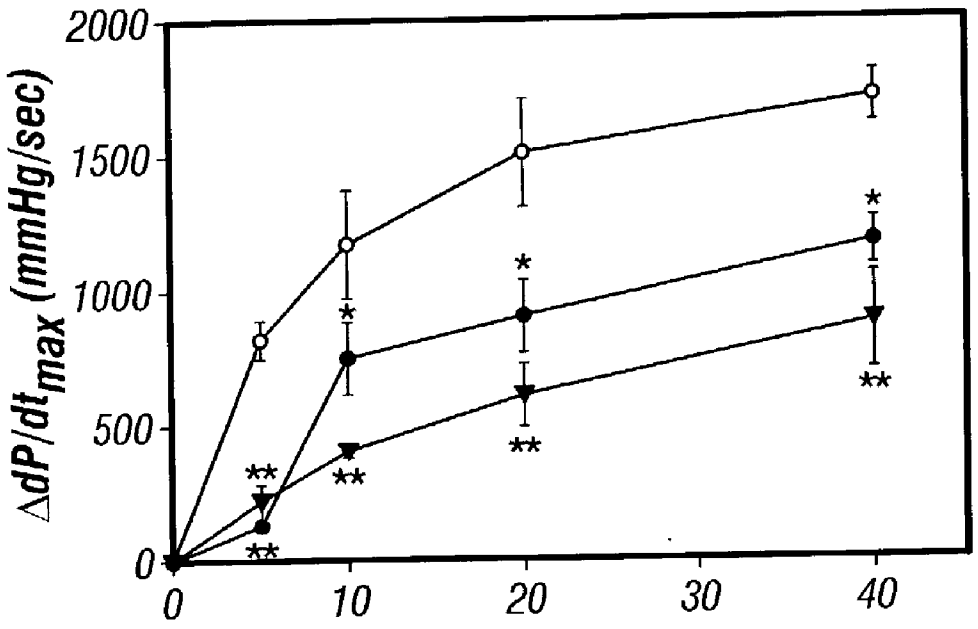
2000
1500
1000
500
0
ΔdP/dtmax (mmHg/sec)
0
10
20
30
40
*
**

FIG. 3A

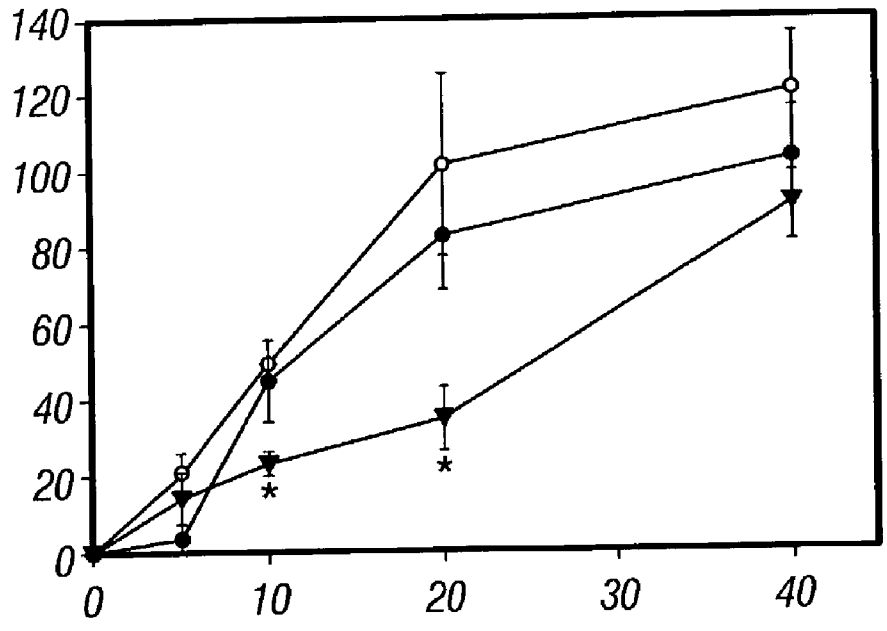
140
120
100
80
60
40
20
0
0
10
20
30
40
*

FIG. 3B

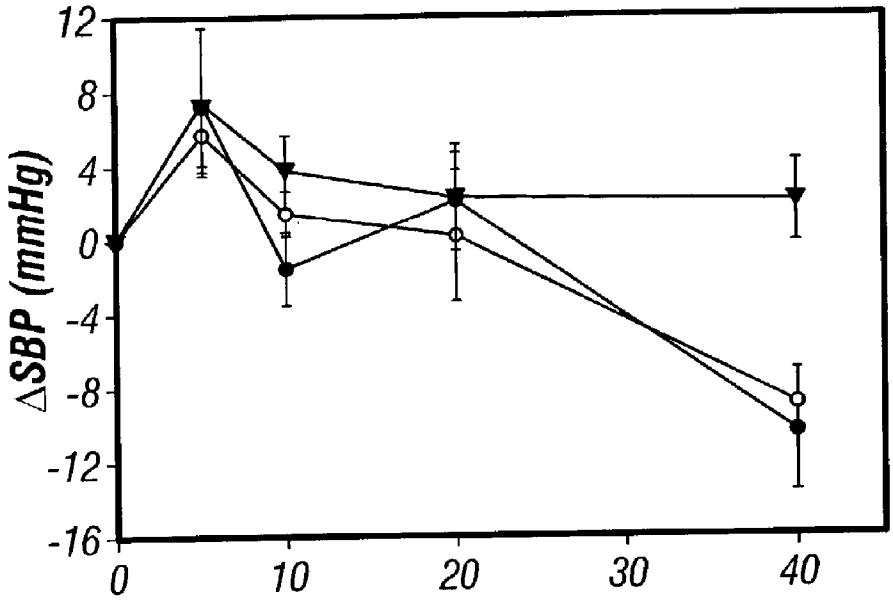
12
8
4
0
-4
-8
-12
-16
ΔSBP (mmHg)
0
10
20
30
40

FIG. 3C

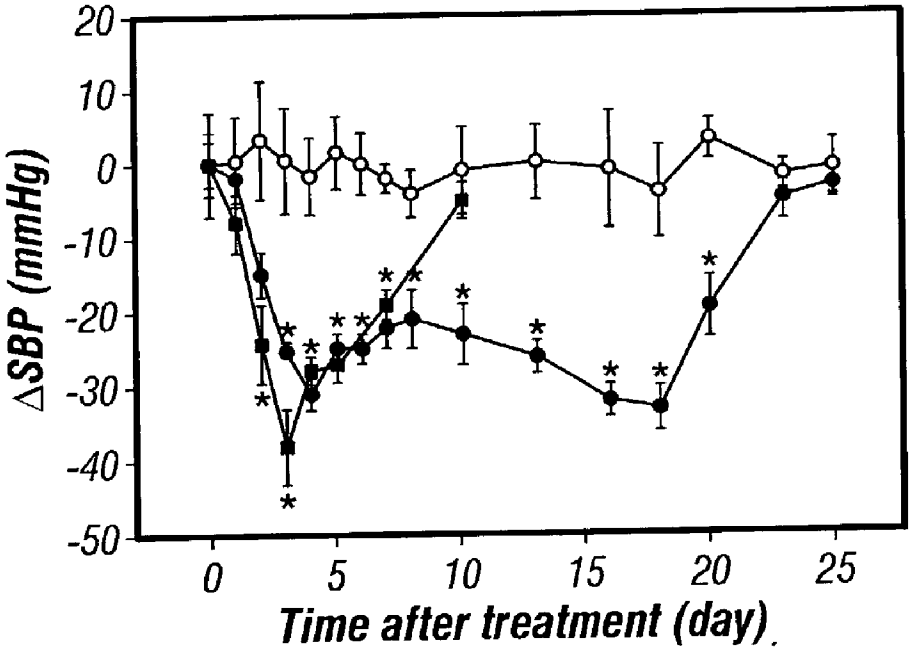
20
10
0
-10
-20
-30
-40
-50
ΔSBP (mmHg)
0
5
10
15
20
25
Time after treatment (day)

FIG. 4

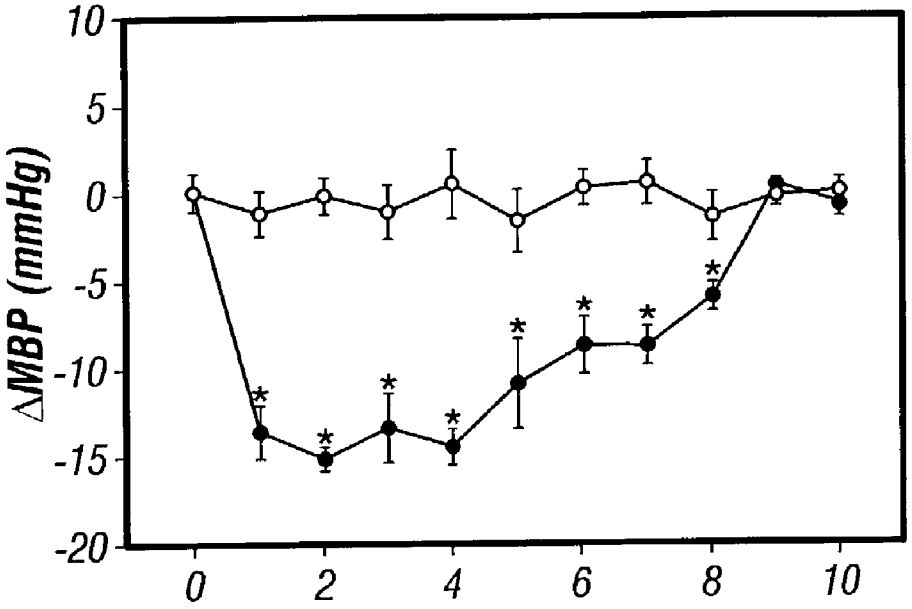
10
5
0
-5
-10
-15
-20
ΔMBP (mmHg)
0
2
4
6
8
10

FIG. 5A

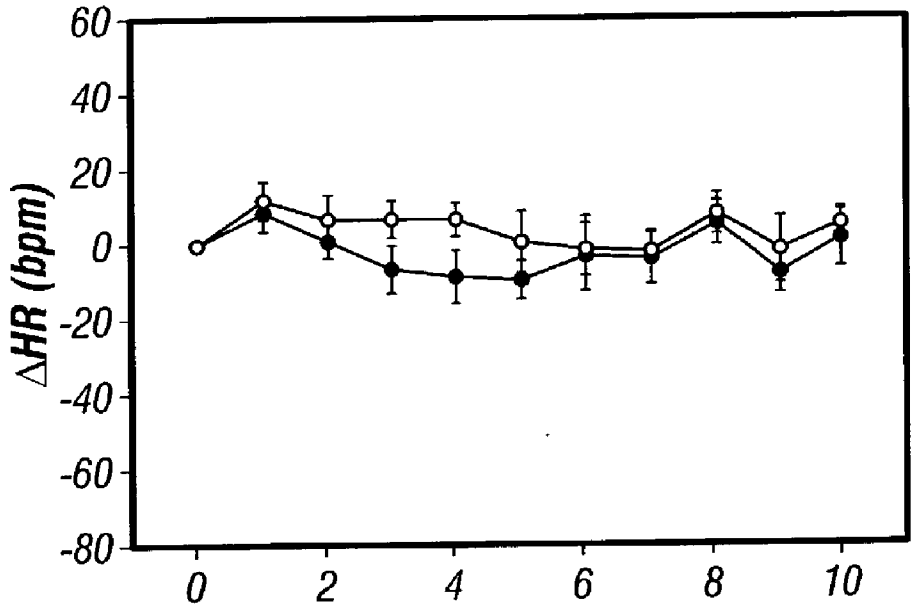
60
40
20
0
-20
-40
-60
-80
ΔHR (bpm)
0
2
4
6
8
10

FIG. 5B

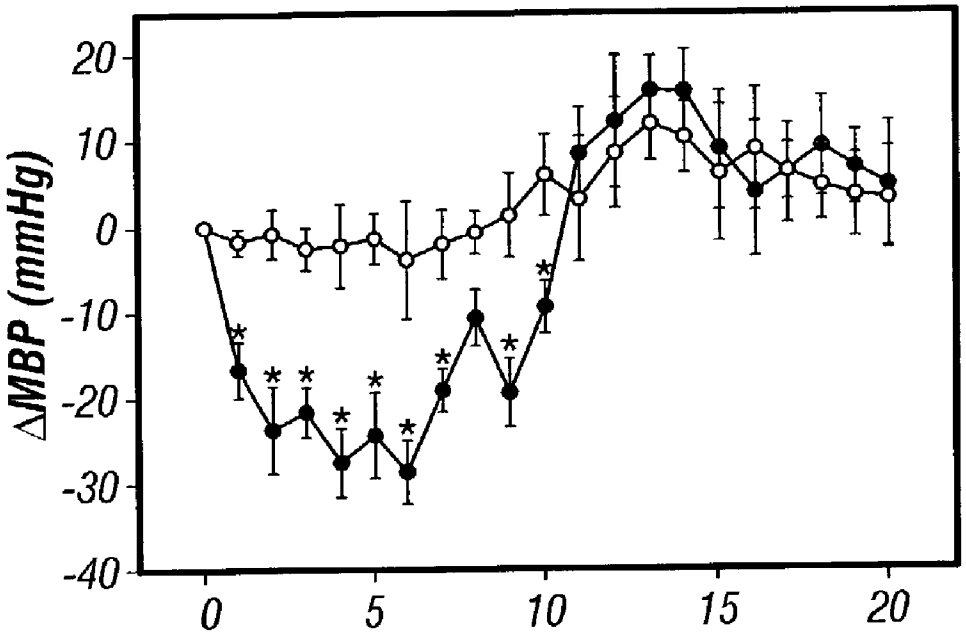
ΔMBP (mmHg)
20
10
0
-10
-20
-30
-40
0
5
10
15
20

FIG. 6A

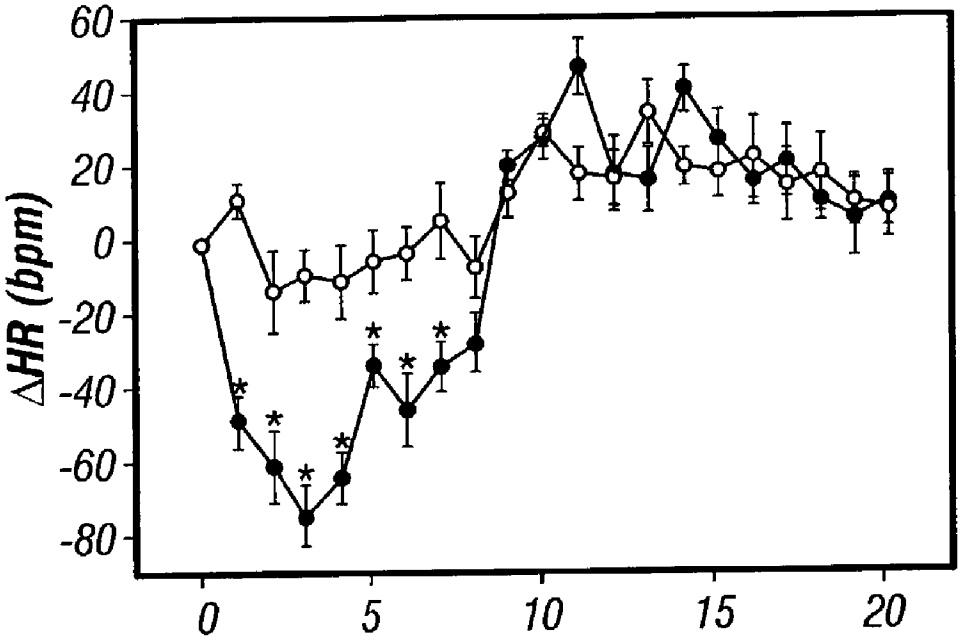
ΔHR (bpm)
60
40
20
0
-20
-40
-60
-80
0
5
10
15
20

FIG. 6B

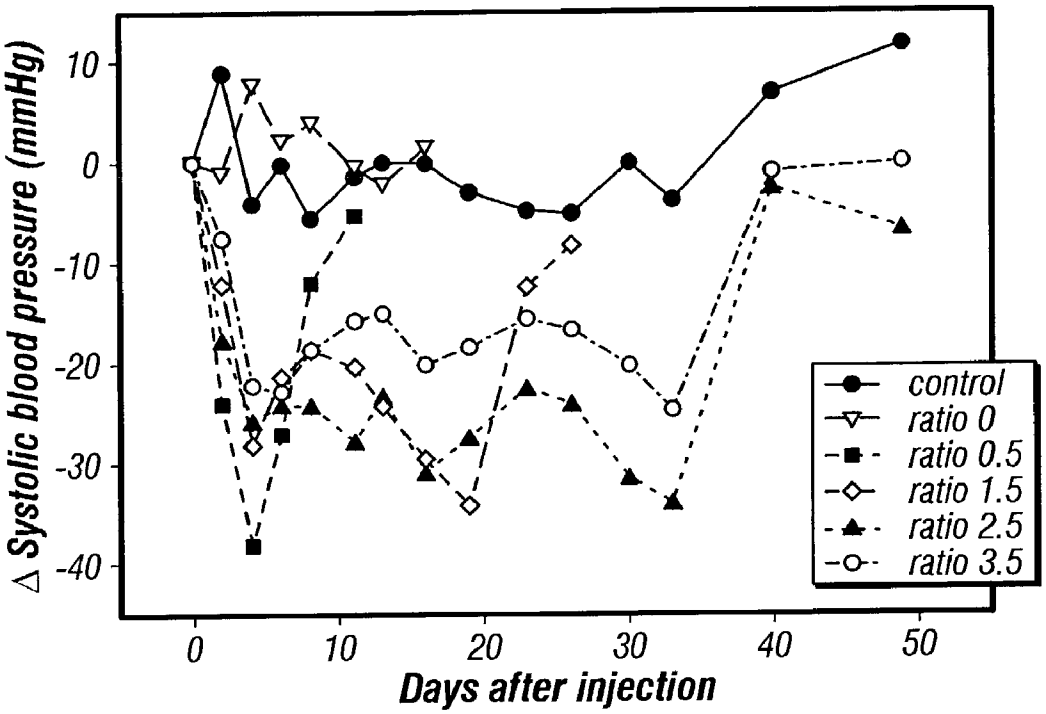
Δ Systolic blood pressure (mmHg)
10
0
-10
-20
-30
-40
0
10
20
30
40
50
Days after injection
control
ratio 0
ratio 0.5
ratio 1.5
ratio 2.5
ratio 3.5

FIG. 7

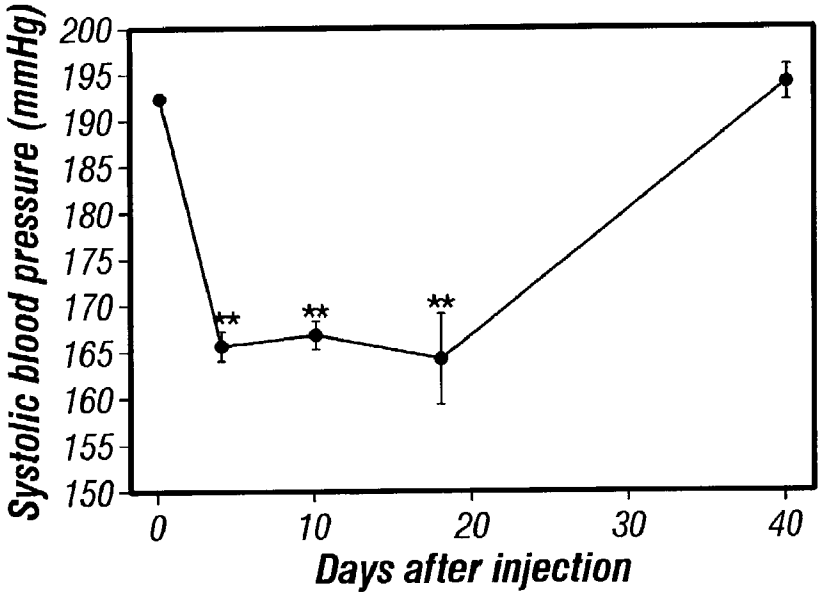
Systolic blood pressure (mmHg)
200
195
190
185
180
175
170
165
160
155
150
**
**
**
0
10
20
30
40
Days after injection

FIG. 8A

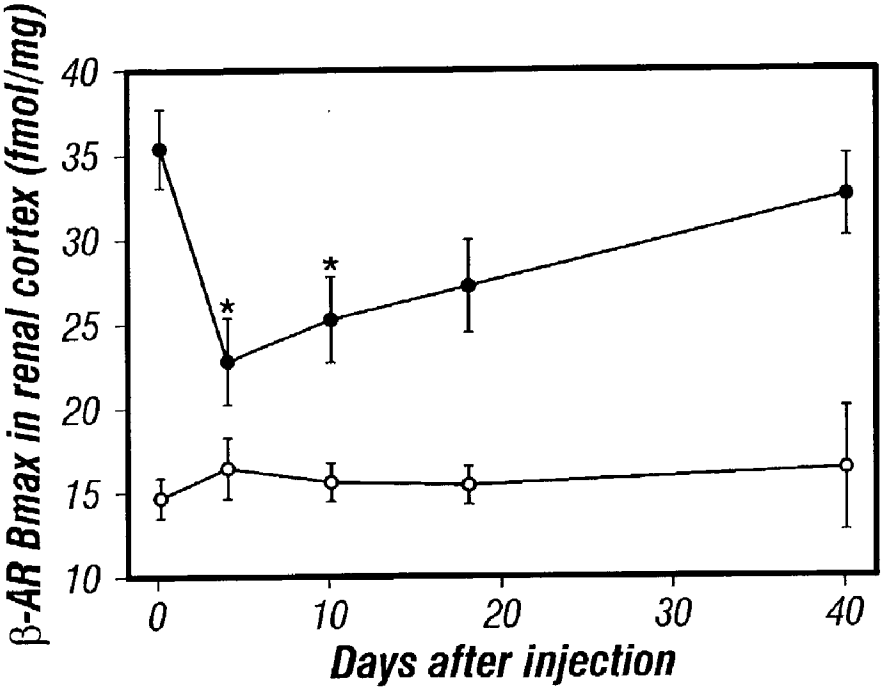
β-AR Bmax in renal cortex (fmol/mg)
40
35
30
25
20
15
10
0
10
20
30
40
Days after injection
*
*

FIG. 8B

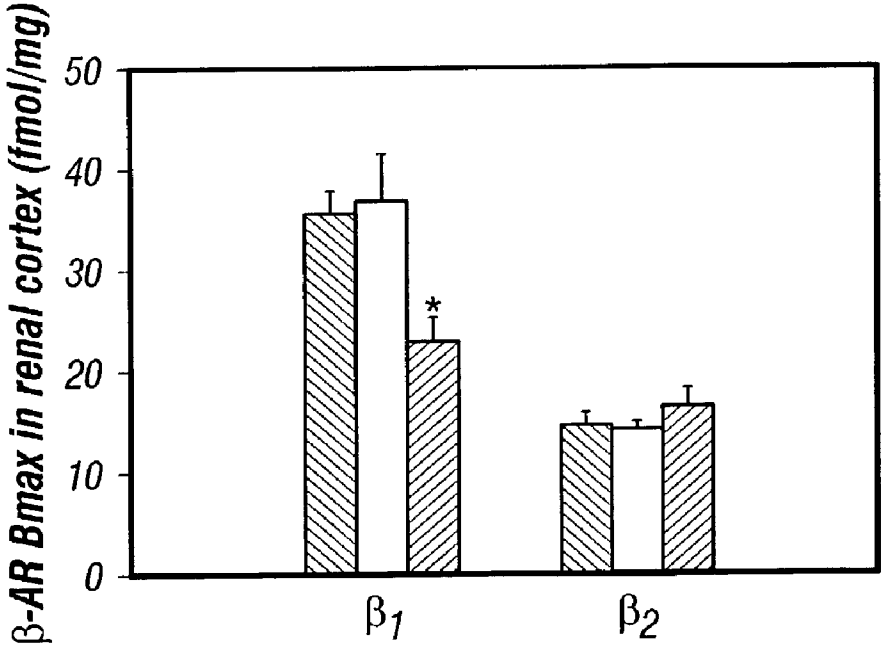
β-AR Bmax in renal cortex (fmol/mg)
50
40
30
20
10
0
*
β1
β2

FIG. 8C

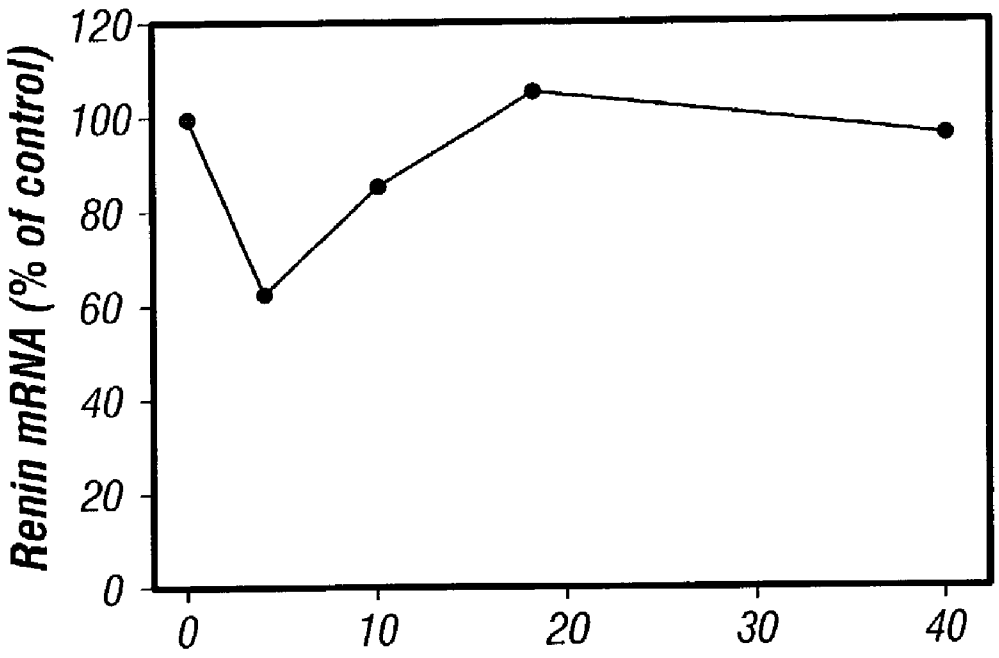
Renin mRNA (% of control)
120
100
80
60
40
20
0
0
10
20
30
40

FIG. 9A

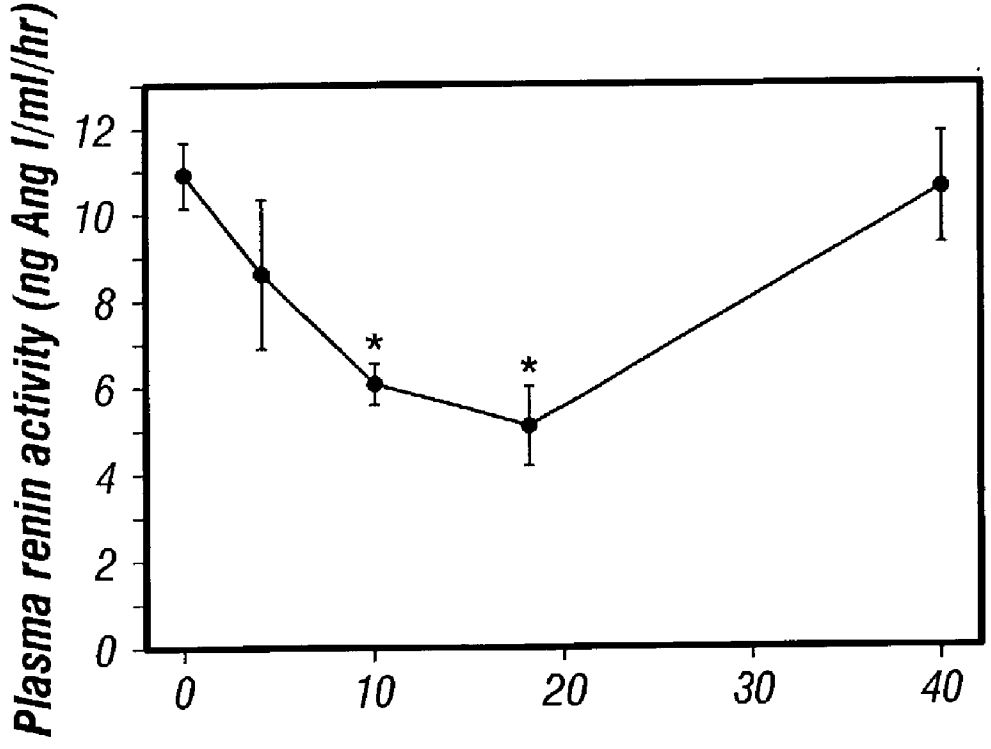
Plasma renin activity (ng Ang I/ml/hr)
12
10
8
6
4
2
0
0
10
20
30
40
*
*

FIG. 9B

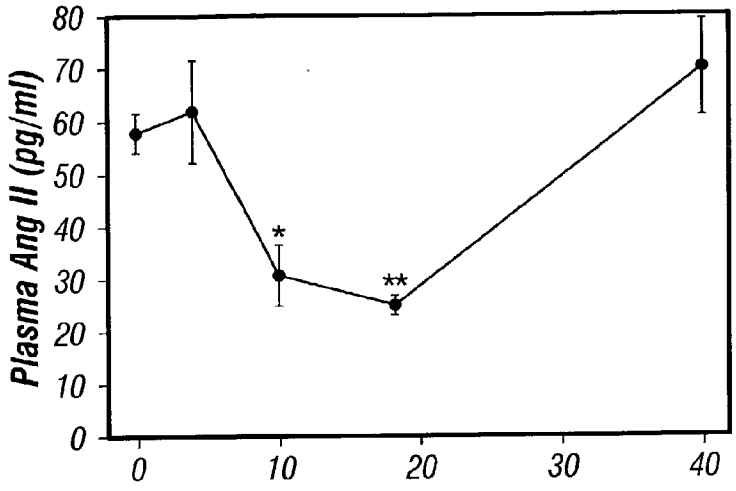
Plasma Ang II (pg/ml)
80
70
60
50
40
30
20
10
0
0
10
20
30
40
*
**

FIG. 9C

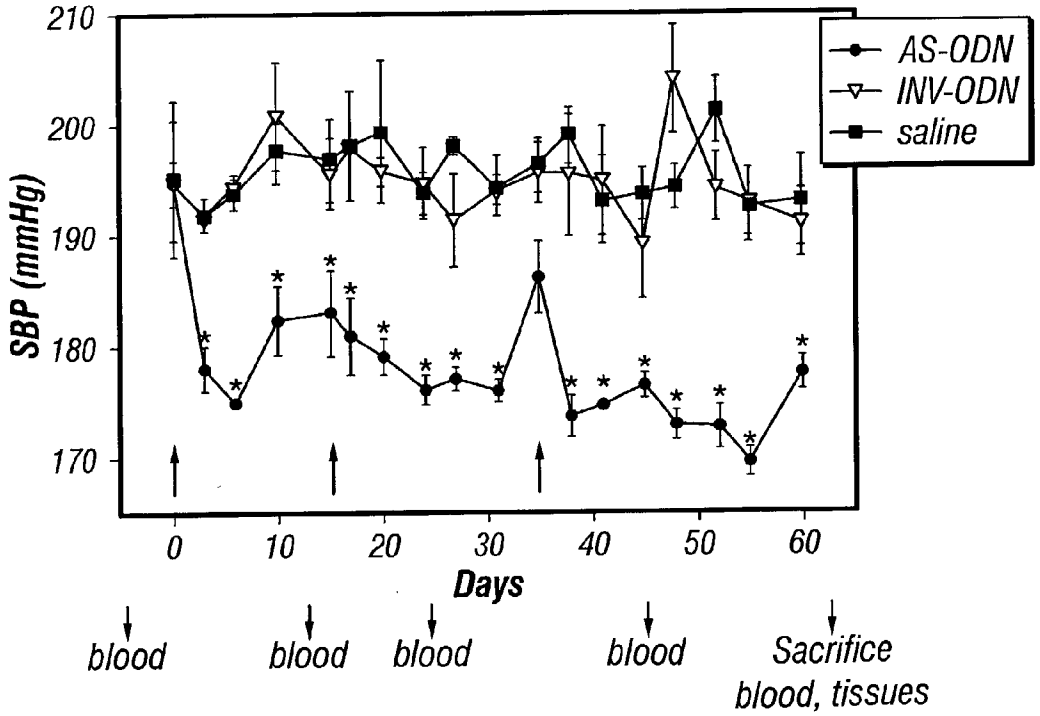
SBP (mmHg)
210
200
190
180
170
0
10
20
30
40
50
60
Days
AS-ODN
INV-ODN
saline
blood
blood
blood
blood
Sacrifice
blood, tissues

FIG. 10

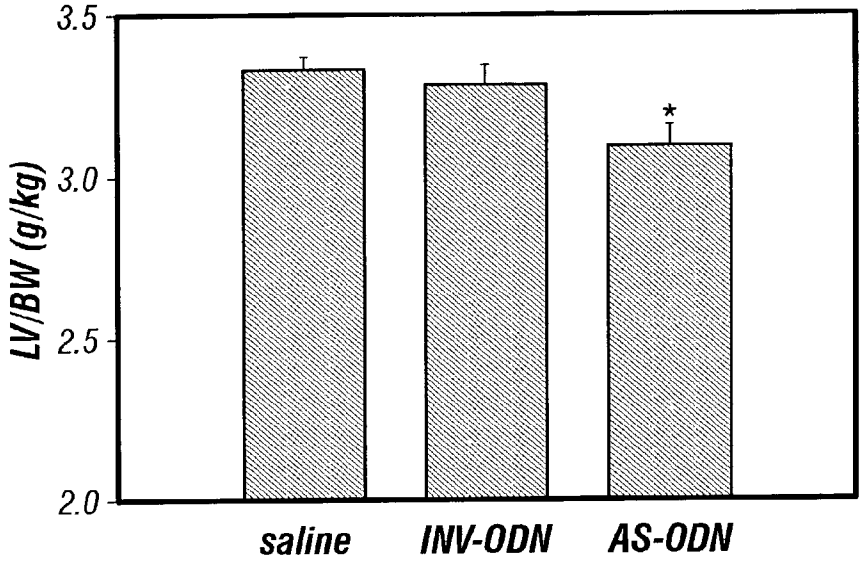

FIG. 11

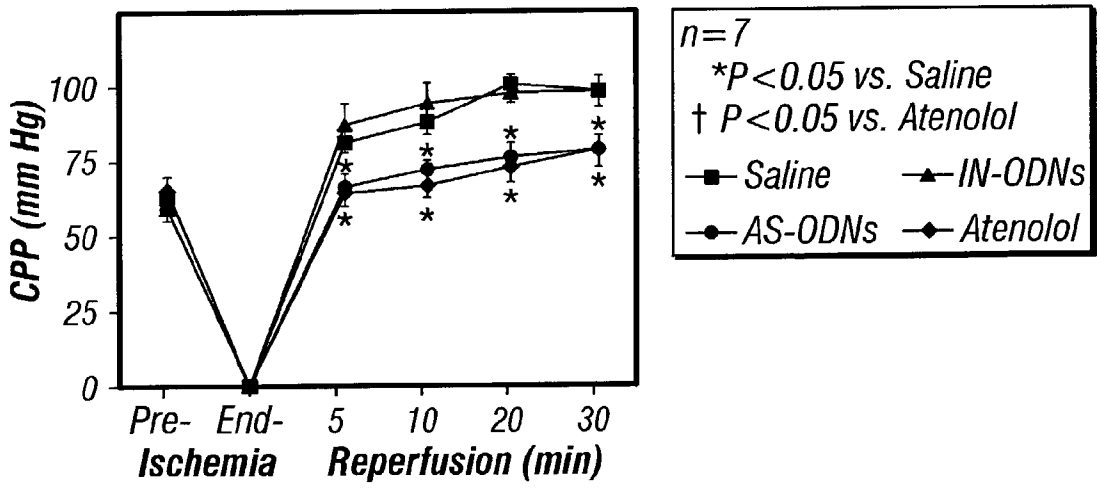

FIG. 12A

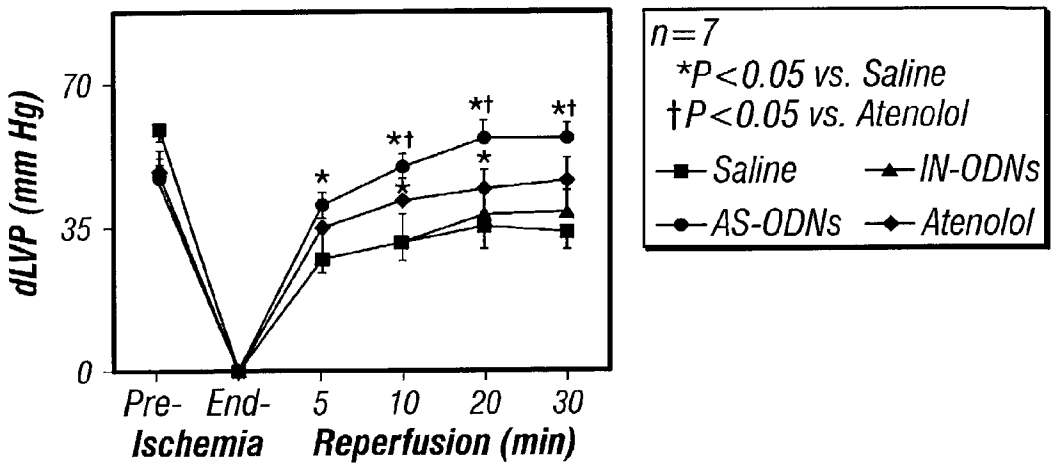

FIG. 12B

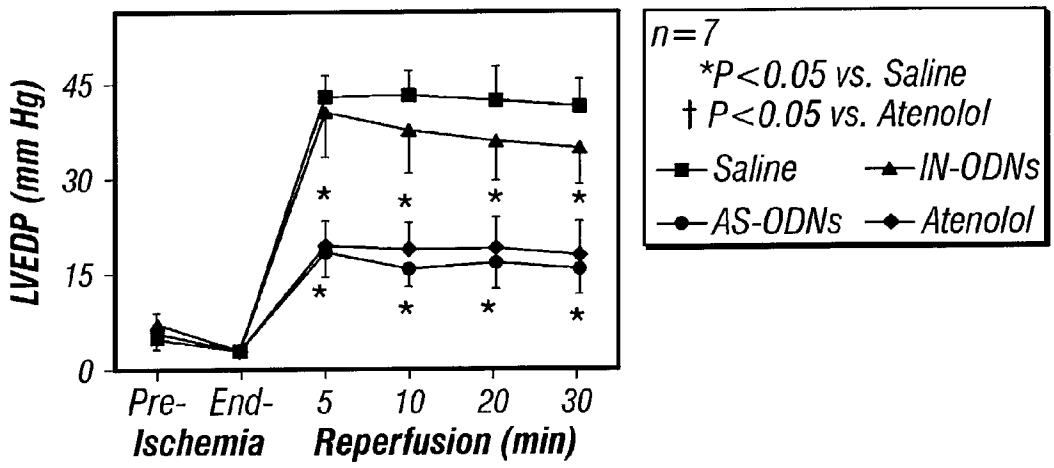

FIG. 12C

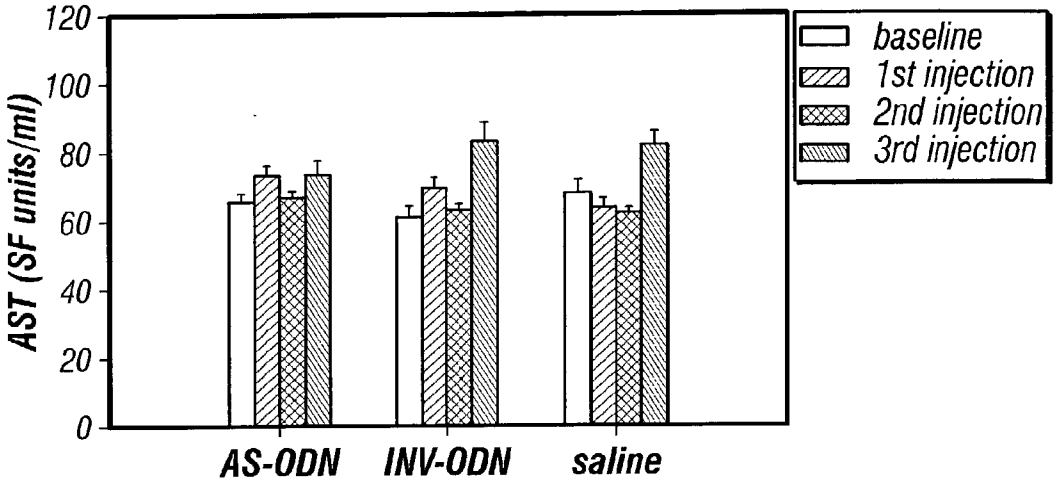

FIG. 13A

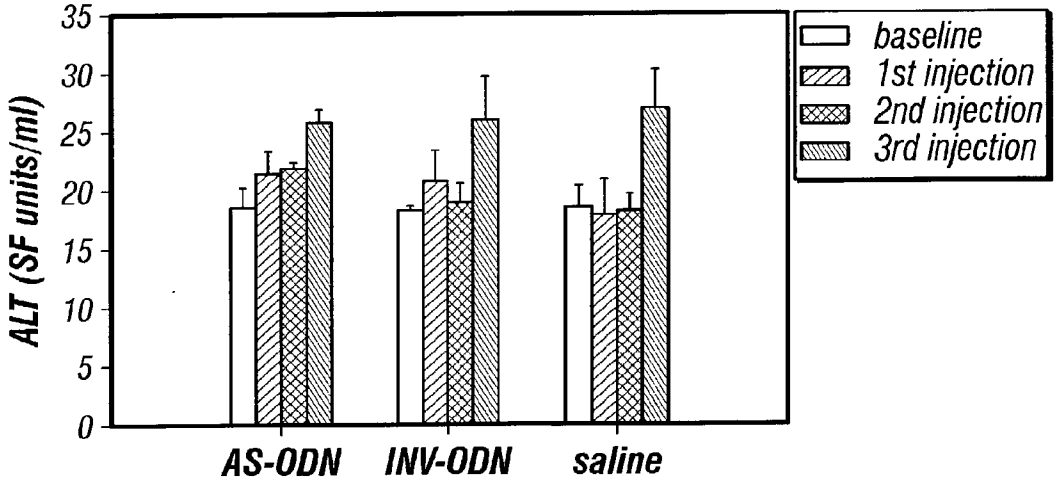

FIG. 13B

ANTISENSE COMPOSITIONS TARGETED TO BETA1-ADRENOCEPTOR-SPECIFIC MRNA AND METHODS OF USE

[0001] The present application is a continuation-in-part of U.S. patent application Ser. No. 09/152,717 filed Sep. 14, 1998, and issued Jul. 11, 2000 as U.S. Pat. No. 6,087,343, and PCT International patent application Serial No. PCT/US99/21007, filed, Sep. 14, 1999, and published Mar. 23, 2000 as WO 00/15783, the entire contents of each of which is specifically incorporated herein by reference in its entirety without disclaimer.

[0002] The United States government has certain rights in the present invention pursuant to Grant HL-27334 from the National Institutes of Health.

1.0 BACKGROUND OF THE INVENTION

[0003] 1.1 Field of the Invention

[0004] The present invention relates generally to the fields of cardiovascular disease and hypertension. More particularly, it concerns antisense oligonucleotide compositions and methods that are useful for reducing hypertension, cardiac hypertrophy, and myocardial ischemia in animals, particularly in mammals such as humans. Specifically, the invention provides antisense oligonucleotide and polynucleotide compositions capable of binding to β_1-adrenoceptor (β_1-adrenergic receptor, β_1-AR)-specific mRNA and inhibiting translation of the β_1-adrenoceptor mRNA, thereby decreasing the number of β_1-AR polypeptides in cells capable of expressing this mRNA. Disclosed are antisense oligonucleotide and peptide nucleic acid compositions, pharmaceutical formulations thereof, and vectors encoding antisense oligonucleotides that specifically bind β_1-AR mRNA and alter expression of the mRNA in a host cell.

[0005] 1.2 Description of Related Art

[0006] 1.2.1 Hypertension

[0007] Hypertension is the result of increased arterial resistance to blood flow and left untreated can lead to various pathological consequences. Hypertension affects approximately 40 million people in the United States. Heart attack (Nicholls et al., 1998), kidney damage (Agodoa, 1998), stroke (Chamorro et al., 1998) and loss of vision (Satllworth and Waldron, 1997) are typical conditions that result from high blood pressure. When blood vessels are subjected to high pressure for extended periods of time, they respond by thickening, vasospasm, and internal build-up of lipids and plaques, a condition known as arteriosclerosis. Arteriosclerosis further causes a decreased blood flow to the kidneys, which respond by releasing the protease renin. An overactive renin-angiotensin system is often implicated in the development of hypertension and cardiovascular disease (Nicholls et al., 1998).

[0008] Hypertension is often called the "silent killer," since half of the population afflicted with high blood pressure are unaware of the condition. Thus, an initial step in combating hypertension is early detection. Following diagnosis, actions must be taken to control the disorder.

[0009] 1.2.2 Treatment of Hypertension

[0010] Currently, four major categories of hypertensive drugs are administered to treat high blood pressure: (1) Diuretics, typically the drug of choice when the abnormal blood pressure is not very high, increase the rate at which the body eliminates urine and salt, resulting in decreased blood pressure by reducing volume (Moser, 1998); (2) β-adrenergic blockers (β-blockers), typically prescribed in combination with diuretics, lower blood pressure and heart rate (Rodgers, 1998); (3) Calcium channel blockers work by preventing the entry of calcium into cells, which reduces vasoconstriction (Rosenthal, 1993); (4) angiotensin converting enzyme (ACE) inhibitors prevent the narrowing and constriction of blood vessels by blocking the production of the vasoconstrictive peptide angiotensin II (AT-II), a product of ACE (Rosenthal, 1993).

[0011] Unfortunately, the short lasting effects of these drugs often require multiple, even daily doses to be therapeutically effective. Poor compliance is a major problem with drug regimens and can lead to a hypertensive crisis if the drug is not taken as scheduled.

[0012] The sympathetic nervous system plays a crucial role in the regulation of blood pressure (BP), mainly through activating α- and β-adrenergic receptors β_1-ARs) in the effector organs, including heart, kidney, and blood vessels. Adrenergic-blocking agents, especially β-blockers, are commonly used in the treatment of hypertension, ischemic heart disease, and arrhythmia (reviewed in Sproat and Lopez, 1991). However, β-blockers cause several side effects, which are usually associated with their central nervous system (CNS) reaction (e.g., sleep disturbance, depression, impotence, dizziness and fatigue) and β_2-adrenergic antagonistic activity (e.g., increase in peripheral vascular resistance, worsening of asthma symptoms). In addition, because of their short half-life (3 to 10 hrs), β-blockers must be taken daily to be effective. Because a cardiovascular disease such as hypertension is a life-lone disorder, longer-lasting treatment without side effects would be desirable.

[0013] Although the precise mechanism underlying the antihypertensive effects of β-blockers remains unclear, it is generally accepted that they antagonize the β_1-AR activity in heart and kidney, decreasing cardiac output and plasma renin activity (Sproat and Lopez, 1991). A new approach to β_1-blockade has been designed that reduces the number of receptors. Antisense oligonucleotides (AS-ODN) or antisense DNA designed specifically against β_1-ARs might represent a new class of β-blockers. Antisense techniques, through a number of mechanisms (Phillips et al., 1997), can effectively downregulate the expression of target proteins. Clinical trials using antisense in targeting AIDS (Akhtar and Rossi, 1996), cancer (Dachs et al., 1997), and other genetic and acquired diseases (Yla, 1997) indicate their potential clinical usefulness. The antisense approach has several potential advantages over β-blockers. First, the specificity of AS-ODNs is based on DNA sequence. Second, AS-ODNs do not have direct CNS effects, because of the negligible transport of these highly polar molecules through the blood-brain barrier (Agrawal et al., 1991). Third, antisense elements tested in different systems produce long-term effects after single treatment (Gyurko et al., 1997). This prolonged effect can be attributed to 2 features of AS-ODN. One is the extended half-life of chemically modified ODN. The half-life 15-20-mer phosphorothioated ODN is 20 to 50 hrs in rats and mice after intravenous injection (Iversen, 1991; Zhang et al., 1995). The other is associated with the nature of antisense inhibition, which provides a delayed yet pro-

longed blockade of target proteins distinct from the direct competitive antagonists currently available.

[0014] 1.2.3 β_1-Adrenoceptors

[0015] β_1-ARs, which are distributed in the heart, kidney and blood vessels, play a role in the physiological control of blood pressure. For many years β-blocker drugs have been used for the treatment of hypertension through a regimen of daily dosing. The mechanism of control of blood pressure is not precisely known but the value of beta-blockers in hypertension control has been underscored by the reports of the Joint National Committee on High Blood Pressure recommending β-blockers as the first line of defense in the treatment of hypertension. Current β-blocker drugs, however, have certain disadvantages, including: (1) they have to be taken daily, or twice a day and compliance is a problem; (2) they have central effects, leading to psychological changes that contribute to the problem of patient compliance; and (3) many of the β-blockers now available are not specific for β_1-ARs and, therefore, can have untoward side effects.

[0016] 1.3 Deficiencies in the Prior Art

[0017] As can be understood from the above, there remains a need for an effective β_1-AR blocker that is highly specific, nontoxic, produces few side effects and does not cross the blood brain barrier to produce psychological changes. Also, a β-blocker that would last several days or weeks would allow patients more flexibility in the regimen of drug dosage by taking drugs infrequently. Thus, the need exists for an effective treatment of cardiac deficiencies (including hypertension, hypertrophy, ischemias, and the like) that circumvents the toxic side effects of existing therapies and provides more specific β_1-AR inhibition with longer acting effects for improved patient compliance. In addition, methods for delivery of antisense oligonucleotides and polynucleotides to a host cell, and in particular, non-invasive administration of specific antisense constructs to a mammal such as a human subject is particularly desirable.

2.0 SUMMARY OF THE INVENTION

[0018] The present invention overcomes these and other limitations in the prior art by providing antisense nucleic acid compounds and compositions comprising them that specifically inhibit or reduce the expression of an mRNA encoding a β_1-adrenoceptor (β_1-AR) polypeptide in a host cell expressing the mRNA. More specifically, the subject invention provides antisense oligonucleotides (AS-ONs) and antisense oligo-peptide nucleic acids (AS-PNAs) that can specifically bind to a mammalian β_1-AR mRNA, resulting in the reduction or inhibition of translation of the messenger ribonucleic acid (mRNA) into β_1-AR polypeptide. Such oligonucleotides and PNA may be readily formulated in pharmaceutically-acceptable vehicles and provided directly to host cells, or administered systemically to mammals that express β_1-AR mRNA to reduce the level of β_1-AR produced in such cells and affected mammals.

[0019] The invention also provides genetic constructs comprising substantially full-length, antisense polynucleotide (AS-PN) sequences operably linked to a suitable promoter that may be used to transform selected cells to endogenously express "antisense" mRNA sequences that are complementary to the native β_1-AR mRNA sequence. When expressed in a suitable host cell, these essentially full length anti-mRNAs specifically bind to the native β_1-AR mRNA produced in the same host cell, and effectively reduce the availability of native β_1-AR mRNA that can serve as a template for the translation machinery of the host cell to produce β_1-AR polypeptide.

[0020] The antisense compounds and the genetic constructs of the present invention may be prepared in a variety of compositions, and may also be formulated in appropriate pharmaceutical vehicles for administration to human or animal subjects to inhibit or significantly reduce the expression of β_1-AR-specific mRNA, and/or to inhibit or significantly reduce the translation of β_1-AR-specific mRNA into functional β_1-AR polypeptide. The antisense compounds of the present invention, and compositions comprising them provide new and useful therapeutics for the treatment, control, and amelioration of symptoms of a variety of cardiovascular disorders including hypertension, ischemia, cardiac hypertrophy, myocardial infarction, cardiac dysfunction, and diseases of the heart, that result from, or are exacerbated by, expression of β_1-AR-specific mRNA in the host cells of the affected mammal. Moreover, pharmaceutical compositions comprising one or more of the nucleic acid compounds disclosed herein, provide significant advantages over existing conventional therapies-namely, (1) their reduced side effects, (2) their increased efficacy for prolonged periods of time, (3) their ability to increase patient compliance due to their ability to provide therapeutic effects following as little as a single dose of the composition to affected individuals.

[0021] Preferred antisense compounds for use in the practice of the present invention are those nucleic acid and peptide nucleic acid sequences that specifically bind to a gene or an mRNA encoding β_1-AR polypeptide and that inhibit the expression or reduce the level of β_1-AR polypeptide in a mammalian host cell that expresses the gene and/or the mRNA.

[0022] The compounds of the invention, and the pharmaceutical formulations thereof, permit the development of methods for treating hypertension, ischemia, cardiac hypertrophy, cardiac dysfunction, or other cardiovascular diseases through the administration of at least one antisense compound that specifically binds to a mammalian β_1-AR-specific gene or mRNA, wherein the binding of the antisense compound to the gene or the mRNA alters, decreases, inhibits, and/or prevents transcription of the β_1-AR-specific mRNA, or, alternatively alters, decreases, inhibits, and/or prevents translation of the β_1-AR-specific mRNA into functional β_1-AR polypeptide in a host cell. As described above, the present methods offer significant advantages over traditional pharmacological modalities involving drugs that block or interfere with activity of the β_1-AR polypeptide (and not the amount of β_1-AR polypeptide). The present methods also avoid many of the untoward side effects of conventional therapies, avoid invasive surgical procedures, and are effective in lower, less frequent dosing regimens. The ability to dose less frequently represents a key aspect of improving patient compliance in dosing and reduces administration costs associated with more frequent dosage regimens.

[0023] In a first embodiment, the invention provides antisense oligonucleotides that specifically bind to a mammalian

β_1-AR-specific gene or a mammalian β_1-AR-specific mRNA in a host cell, and alter the expression of, or quantity of, β_1-AR polypeptide produced in the cell.

[0024] In a second embodiment, the invention provides antisense peptide nucleic acids that specifically bind to a mammalian β_1-AR-specific gene or a mammalian β_1-AR-specific mRNA in a host cell, and alter the expression of, or quantity of, β_1-AR polypeptide produced in the cell.

[0025] In a third embodiment, the invention provides antisense polynucleotides that specifically bind to a mammalian β_1-AR-specific gene or to a mammalian β_1-AR-specific mRNA in a host cell, and alter the expression of, or quantity of, β_1-AR polypeptide produced in the cell. The antisense polynucleotides represent full-length, or substantially full-length sequences that are complementary to a mammalian β_1-AR-specific mRNA, and that when present in a cell that expresses a mammalian β_1-AR-specific mRNA, will specifically bind to the β_1-AR-specific mRNA, thereby reducing the availability of functional mRNA in the cell from which the cellular protein machinery can translate functional β_1-AR polypeptide. Preferably, these full-length, or substantially full-length polynucleotides are provided to a cell via a genetic construct that comprises a promoter capable of expressing the complementary mRNA in a selected host cell. Such genetic constructs may be provided to suitable host cells using any one of the conventional gene therapy modalities known to those of skill in the art, such as, for example by one or more of the viral, retroviral, adenoviral, or adenoassociated viral constructs commonly exploited for the delivery and expression of heterologous polynucleotides.

[0026] In a fourth embodiment, the invention provides compositions that comprise one or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compounds and a suitable diluent, carrier, or pharmaceutical vehicle.

[0027] In a fifth embodiment, the invention provides pharmaceutical formulations that comprise one or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compounds and at least one antihypertensive pharmaceutical compound.

[0028] In a sixth embodiment, the invention provides pharmaceutical compositions that comprise at least two or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compounds.

[0029] In a seventh embodiment, the invention provides pharmaceutical compositions that comprise at least two or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compounds in combination with at least one antihypertensive pharmaceutical compound.

[0030] In an eighth embodiment, the invention provides therapeutic kits and medicaments that comprise at least one or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compositions in combination with instructions for using the compositions in the treatment of mammalian cardiac dysfunction or disease. Alternatively, the invention provides kits that comprise at least one or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compositions in combination with instructions for using the compositions in the preparation of genetic constructs for the development of suitable gene therapy vectors. Likewise, the invention provides kits that comprise at least one or more of the disclosed antisense oligonucleotides, polynucleotides, and peptide nucleic acid compositions in combination with instructions for using the compositions in the formulation of multi-drug "cocktails" for the treatment of one or more pathological conditions.

[0031] The invention also provides methods for the treatment of one or more cardiac diseases or dysfunctions employing one or more of the antisense compounds or compositions as described herein.

[0032] In each of the embodiments described herein, the oligonucleotide, polynucleotide, or peptide nucleic acid compound comprises a sequence region least 9 to about 35 bases in length, wherein the oligonucleotide specifically binds to a portion of mRNA expressed from a gene encoding a mammalian β_1-AR polypeptide, and further wherein binding of the oligonucleotide to the mRNA is effective in decreasing the activity of β_1-AR in a host cell expressing the mRNA.

[0033] 2.1 Oligonucleotide and OLIGO-PNA Compounds

[0034] In oligonucleotide and oligo-peptide nucleic acid embodiments, the oligonucleotide or PNA preferably consists of a sequence of from about nine to about 35 nucleotides in length, and preferably comprises a sequence of at least nine, at least ten, at least eleven, at least twelve, at least thirteen at least fourteen, or at least fifteen contiguous bases from any one of the sequences disclosed in SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, and SEQ ID NO:28, as well as the smaller n-mer sequences illustrated in SEQ ID NO:29 through SEQ ID NO:186.

[0035] Alternatively, the oligonucleotide or PNA preferably consists of a sequence of from about nine to about 35 nucleotides in length, and preferably comprises a sequence of at least sixteen, at least seventeen, at least eighteen, at least nineteen, at least twenty, at least twenty-one, at least twenty-two, at least twenty-three, at least twenty-four, or at least twenty-five contiguous nucleotides from any one of the sequences disclosed in SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO: 1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53.

[0036] Alternatively, the oligonucleotide or PNA preferably consists of a sequence of from about nine to about 35

nucleotides in length, and preferably comprises a sequence of at least twenty-six, at least twenty-seven, at least twenty-eight, at least twenty-nine, at least thirty, at least thirty-one, at least thirty-two, at least thirty-three, or at least thirty-four contiguous nucleotides from any one of the sequences disclosed in SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11. SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53.

[0037] Alternatively, the oligonucleotide or PNA may consist of a sequence of from about nine to about 35 nucleotides in length, and may comprise the entire sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53.

[0038] The oligonucleotide or PNA compound may consist essentially of the sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or any one or more of the smaller n-mer sequences illustrated in SEQ ID NO:54 through SEQ ID NO:186.

[0039] The oligonucleotide or PNA compound may also consist of the sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or any one or more of the smaller n-mer sequences illustrated in SEQ ID NO:54 through SEQ ID NO:186.

[0040] As described above, the length of the preferred oligonucleotide or PNA compounds preferably will be on the order of from about nine to about 35 or so nucleotides. As such, in addition to those compounds that are nine nucleotides in length, and those compounds that are 35 nucleotides in length, all intermediate size compounds are also contemplated to be useful in the practice of the present invention. Thus, oligonucleotide or PNA compounds that are ten nucleotides in length, eleven nucleotides in length, twelve nucleotides in length, thirteen nucleotides in length, fourteen nucleotides in length, fifteen nucleotides in length, sixteen nucleotides in length, seventeen nucleotides in length, eighteen nucleotides in length, nineteen nucleotides in length, twenty nucleotides in length, twenty-one nucleotides in length, twenty-two nucleotides in length, twenty-three nucleotides in length, twenty-four nucleotides in length, twenty-five nucleotides in length, twenty-six nucleotides in length, twenty-seven nucleotides in length, twenty eight nucleotides in length, twenty-nine nucleotides in length, thirty nucleotides in length, thirty-one nucleotides in length, thirty-two nucleotides in length, thirty-three nucleotides in length, and thirty-four nucleotides in length are also contemplated to fall within the scope of the present disclosure. In fact, the preferred oligonucleotide or PNA compounds may also be slightly shorter than the preferred range, that is, they may be about six or about seven or about eight nucleotides in length, or they may even be slightly longer than the preferred range (i.e., they may be about thirty-six or about thirty-seven or even about thirty-eight or so nucleotides in length), and may still function to reduce the level of β_1-AR polypeptide in a cell, and thus, effective in the treatment of cardiac disorders resulting from an elevated level of β_1-AR polypeptide.

[0041] Likewise, there is no obligate requirement that the antisense compounds be exactly 100% complementary to a given target sequence on the mammalian β_1-AR mRNA. Nor is there an obligate requirement that the antisense compounds be exactly 100% identical to one of the sequences disclosed in SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or any one or more of the smaller

n-mer sequences illustrated in SEQ ID NO:54 through SEQ ID NO:186. In fact, the only requirement is that the oligonucleotide or PNA compound have sufficient homology to the gene or the mRNA encoding β_1-AR polypeptide so that upon specifically binding to the complementary region, a reduction in either the transcription of the β_1-AR gene and/or a reduction in the translation of β_1-AR polypeptide from the mRNA is observed. Therefore, the compounds may have 4 or fewer, 3 or fewer, 2 or fewer, or even 1 mismatch from the target sequence to which it specifically binds, and may still be sufficiently active to cause a reduction in β_1-AR polypeptide within the host cell. Thus, in addition to those compounds disclosed above that comprise sequences that are 100% identical to the sequences disclosed in SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or any one or more of the smaller n-mer sequences illustrated in SEQ ID NO:54 through SEQ ID NO:186, the invention also encompasses antisense compounds that comprise sequence regions that are about 99%, about 98%, about 97%, about 96%, about 95%, about 94%, about 93%, about 92%, about 91%, or even about 90% identical to a portion of one of those sequences, so long as the resulting degenerate nucleotide sequence retains sufficient homology so that it specifically binds to the gene or mRNA encoding β_1-AR polypeptide.

[0042] 2.2 Polynucleotide and Poly-PNA Compounds

[0043] In certain embodiments, the polynucleotide comprises at least 9 contiguous bases from SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53. In other aspects of the invention, the polynucleotide comprises DNA, RNA, PNA, or preferably a derivatized or modified polynucleotide, including phosphorothioate derivates and the like.

[0044] Preferably the antisense polynucleotide compound comprises a sequence that is substantially the full-length complement of the β_1-AR mRNA, wherein binding of the substantially full-length complementary sequence to the β_1-AR mRNA is effective in reducing the translation of the mRNA into the β_1-AR polypeptide. Preferably the substantially full-length complementary sequence is comprised within a suitable vector that may be delivered to appropriate host cells. The vector preferably comprises a nucleic acid segment that is operably linked to a promoter sequence that expresses the complementary antisense sequence in the host cell. This complementary near full-length sequence is then able to specifically bind to the substantially full length β_1-AR mRNA and thereby prevent or substantially reduce the translation of the mRNA into β_1-AR polypeptide. The term substantially full-length is meant to include those sequences that comprise a sequence that is complementary to a region that is at least about 80% or more of the native β_1-AR-specific mRNA. Thus, for a β_1-AR mRNA that is 1000 nucleotides in length, a suitable complementary substantially full-length complement is a sequence that is complementary to a region of at least 800 nucleotides of the particular β_1-AR mRNA. Likewise, all sequences that are greater than 80% of the full-length sequences (i.e. those that are at least 81% full length, at least 82% full length, at least 83% full length, at least 84% full length, at least 85% full length, at least 86% full length, at least 87% full length, at least 88% full length, at least 89% full length, at least 90% full length, at least 91% full length, at least 92% full length, at least 93% full length, at least 94% full length, at least 95% full length, at least 96% full length, at least 97% full length, at least 98% full length, and at least 99% full length are also contemplated to fall within the scope of the present disclosure.

[0045] 2.3 Compositions Comprising a β_1-AR Antisense Compound and a Second Cardiac Therapeutic Agent

[0046] When desirable, the clinician may combine two or more of the oligonucleotide, polynucleotide, or PNA compounds disclosed above to provide an antisense "cocktail" to effect a more substantial reduction in the levels of β_1-AR polypeptide. Likewise, the patient or animal to be treated may benefit from a combination therapy involving one or more of the oligonucleotide, polynucleotide, or PNA compounds disclosed herein and at least one anti-hypertensive or other cardiac therapy medicament.

[0047] For example, the composition may compris at least a first anti-hypertensive agent. In particular embodiments, the anti-hypertensive agent is selected from the group consisting of captopril (Captopril®), enalapril (Vasotec®), ramipril (Altace®), fosinopril (Monopril®), lisinopril (Prinivil®, Zestril®), quinapril (Accupril®), benazepril (Lotensin®), trandolapril (Mavik®), and moexipril (Univasc®). In other embodiments, the anti-hypertensive agent is selected from the group of angiotensin receptor blockers consisting of candesartan (Atacand®), losartan (Cozaar® and Hizaar®), valsartan (Diovan®), and irbesartan (Avapro®).

[0048] In still other embodiments, the anti-hypertensive agent is selected from the group of diuretic consisting of dichlorphenamide (Daramide®), spironolactone (Aldactazide®), hydrochlorothiazide (Microzide® and Dyazide®), triamterene (Maxzide®), amiloride (Midamor® and Moduretic®), torsemide (Demadex®), ethacrynice acid (Edecrin®), furosemide (Lasix®), hydroflumethiazide (Diucardin®), chlorothiazide (Diuril®), methylclothiazide (Enduron®), polythiazide (Renese®), chlorthalidone (Thalitone®) and metolazone (Zaroxolyn®).

[0049] In still further embodiments, the anti-hypertensive agent is selected from the group of calcium channel blockers consisting of nifedepine (Adalat® and Procardia®), verapamil (Isoptin®, Verelan®, Calan® and Covera®), nicardipine (Cardene®), diltiazem (Tiazac®, Cardizem® and Dilacor®), isradipine (DynaCirc®), nimodipine (Nimotop®), amlodipine (Norvase®), felodipine (Plendil®), misoldipine (Sular®), and bepridil (Vasocor®).

[0050] In certain preferred embodiments, the composition may further comprise one or more pharmaceutically acceptable vehicles, exemplified by, but not limited to, liposomes, lipid particles, lipid vesicles, nanoparticles, microparticles, nanocapsules, nanospheres, and sphingosomes to facilitate administration of the antisense composition(s) to the affected patient or animal.

[0051] In certain aspects of the invention, the antisense composition of the present invention is specific for an mRNA encoding the human β_1-AR polypeptide. In particular embodiments, the host cell is a mammalian host cell. In certain preferred embodiments of the invention, the host cell is a human cell. In other preferred aspects, the host cell is comprised within a human.

[0052] 2.4 Compositions Comprising a β_1-AR Antisense Compound and a Second Antisense Targeted to Another RAS Pathway Component

[0053] The present invention also provides compositions that comprise at least a first antisense oligonucleotide, PNA, or polynucleotide specific for a mammalian β_1-AR-specific mRNA as described above, and at least a second antisense compound specific for a mammalian renin, angiotensin, angiotensinogen, angiotensin type 1 (AT-1) receptor mRNA, or an ACE polypeptide.

[0054] In certain aspects of the invention, the second antisense compound is specific for a mammalian angiotensinogen mRNA, or an mRNA that encodes one or more RAS pathway-specific enzymes, such as a mammalian ACE polypeptide. In other aspects, the second antisense compound is specific for an mRNA that encodes a transcriptional factor.

[0055] Therapeutic combinations of three or more antisense compound are also provided. These cocktail therapies may comprise at least two antisense compounds specific for a mammalian β_1-AR-encoding gene or mRNA, and at least a third antisense compound specific for the same mRNA, or alternatively, an mRNA encoding another polypeptide in the RAS pathway as described above. The third antisense compound may be specific for renin, AT-1 receptor, ACE, or angiotensinogen, or another gene or mRNA involved in biochemical pathways involved in producing or regulating blood pressure and/or causing or contributing to hypertension, ischemia, cardiac hypertrophy, or other cardiac dysfunction in an affected animal. Alternatively, the constructs may even be specific for one or more particular transcription factor(s) that regulate one or more genes involved in producing hypertension, ischemia, cardiac hypertrophy, or other cardiac dysfunction in a mammal. Such combined therapy approached using two or more antisense oligonucleotides are particularly desirable where enhanced or synergistic activity towards treating hypertension ischemia, cardiac hypertrophy, or other cardiac dysfunction is achieved.

[0056] The invention further provides a method for reducing expression of a gene encoding mammalian β_1-AR polypeptide in a host cell, the method comprising providing to the host cell an amount of an antisense oligonucleotide, polynucleotide, or peptide nucleic acid that specifically binds to an mRNA encoding the β_1-AR polypeptide, effective to reduce the amount of β_1-AR polypeptide in the cell.

[0057] Additionally, the invention provides a method for reducing the number of mammalian β_1-AR polypeptides in a cell, the method comprising introducing into the cell at least a first antisense oligonucleotide, polynucleotide, or peptide nucleic acid that specifically binds to all, substantially-all, or a portion of the mRNA expressed from a gene encoding a mammalian β_1-adrenoceptor polypeptide, and further wherein binding of the first antisense oligonucleotide, polynucleotide, or peptide nucleic acid to the mRNA is effective in reducing the number of mammalian β_1-AR polypeptides in the host cell expressing the mRNA.

[0058] The invention also provides a method for decreasing or treating hypertension in an animal, the method comprising administering to the animal an effective amount of at least a first antisense oligonucleotide, polynucleotide, or peptide nucleic acid, wherein the compound specifically binds to all, substantially all, or a portion of the mRNA expressed from a gene encoding a mammalian β_1-AR polypeptide, and further wherein binding of the compound to the mRNA is effective in decreasing the number of such polypeptides in a host cell expressing the mRNA.

[0059] The invention additionally provides a method for treating a disease associated with elevated β_1-AR activity in a mammal, the method comprising administering to the animal an effective amount of at least a first antisense oligonucleotide, polynucleotide, or peptide nucleic acid compound wherein the compound specifically binds to all, substantially all, or a portion of the mRNA expressed from a gene encoding a mammalian β_1-AR polypeptide, and further wherein binding of the compound to the mRNA is effective in decreasing the receptor activity or receptor number in a host cell expressing the mRNA, such that a decrease in β_1-adrenoceptor activity is effected, thereby resulting in amelioration of the disease that results from, or is exacerbated by, an elevated level of β_1-AR activity in the affected animal.

[0060] The invention also provides a method for treating a cardiac hypertrophic or ischemic disease that is associated with, that results from, or is exacerbated by, the presence of, or an elevation in, β_1-adrenoceptor polypeptides in the affected animal. This method generally involves the administration to the animal of one or more compositions that comprise at least a first antisense oligonucleotide, polynucleotide, or peptide nucleic acid compound, wherein the compound specifically binds to all, substantially all, or a portion of the mRNA expressed from a gene encoding a mammalian β_1-AR polypeptide in an amount, and for a time sufficient to decrease the number, amount, or activity of the β_1-adrenoceptor polypeptide in a host cell expressing the mRNA.

[0061] Further provides are kits for treating hypertension in a human comprising: (a) a pharmaceutically-acceptable formulation comprising t least a first oligonucleotide of at least 9 to about 35 bases in length, wherein the oligonucleotide specifically binds to a portion of mRNA expressed from a gene encoding a mammalian β_1-AR polypeptide, and further wherein binding of the oligonucleotide to the mRNA

is effective in decreasing the activity, amount, or number of receptor polypeptides in a host cell expressing the mRNA; a pharmaceutical excipient; and (b) instructions for using the kit.

[0062] In addition to methods involving the delivery of exogenous oligonucleotide compositions to a host cell, or administration of such compositions to an animal in a therapeutic pharmaceutical formulation, the present invention also concerns gene therapy methods for introducing into a host cell a DNA construct that is transcribed by the cell machinery to give rise to an antisense RNA molecule that specifically binds to a portion of an mRNA encoding a mammalian β_1-AR polypeptide. In a preferred embodiment, such therapies may be accomplished through the use of viral vectors, such as retro-, adeno- or adeno-associated viruses as described hereinbelow.

[0063] Regulation of expression of a gene encoding a mammalian β_1-AR polypeptide in a mammalian cell genomes may also be achieved by integration of a gene under the transcription control of a promoter which is functional in the host and in which the transcribed strand of DNA is complementary to the strand of DNA that is transcribed from the endogenous β_1-AR-specific polynucleotide sequence(s) one wishes to regulate. The integrated gene, referred to as an antisense gene, provides an RNA sequence capable of binding to naturally existing RNAs, exemplified by mammalian β_1-AR polypeptide-specific mRNA, and inhibiting its expression, where the anti-sense sequence may bind to the coding, non-coding, or both, portions of the RNA. The antisense construction may be introduced into the animal cell in a variety of ways and be integrated into the animal genome for inducible or constitutive transcription of the antisense sequence.

[0064] Another aspect of the invention provides a pharmaceutical composition useful for inhibiting expression of mammalian β_1-AR-specific mRNA comprising a pharmaceutical carrier and one or more antisense oligonucleotides of the present invention that specifically bind to and reduce expression of the specific mRNA. Another aspect of the invention provides a method for treating hypertension in a human comprising administering to a subject an effective amount of at least one oligonucleotide composition as described herein, in an amount effective to reduce hypertension in the human, or to ameliorate the degree or extent of hypertension in the patient.

[0065] 2.5 Definitions

[0066] The term "β_1-AR" refers to polypeptides having amino acid sequences which are substantially similar to the native mammalian β_1-AR polypeptide amino acid sequences and which are biologically active and/or which cross-react with β_1-AR polypeptide-specific antibodies raised against a mammalian β_1-AR polypeptide or peptide fragment thereof.

[0067] The term "β_1-AR" also includes analogs of mammalian β_1-AR polypeptides that exhibit at least some biological activity in common with native mammalian β_1-AR polypeptides.

[0068] Furthermore, those skilled in the art of mutagenesis will appreciate that other analogs, as yet undisclosed or undiscovered, may be used to construct mammalian β_1-AR polypeptide analogs or β_1-AR fusion proteins or identify β_1-AR-related mRNAs and/or genes using well-known molecular biology techniques, including those described herein. Oligonucleotides complementary to mammalian β_1-AR polypeptide-encoding mRNAs form the heart of the present invention, especially human β_1-AR polypeptide-encoding mRNAs.

[0069] The oligonucleotides (or "ODNs" or "polynucleotides" or "oligos" or "oligomers" or "n-mers") of the present invention are preferably deoxyoligonucleotides (i.e. DNAs), or derivatives thereof; ribo-oligonucleotides (i.e. RNAs) or derivatives thereof; or peptide nucleic acids (PNAs) or derivatives thereof.

[0070] The term "substantially complementary," when used to define either amino acid or nucleic acid sequences, means that a particular subject sequence, for example, an oligonucleotide sequence, is substantially complementary to all or a portion of the selected sequence, and thus will specifically bind to a portion of an Mrna encoding a mammalian β_1-AR polypeptide. As such, typically the sequences will be highly complementary to the Mrna "target" sequence, and will have no more than 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 base mismatches throughout the complementary portion of the sequence. In many instances, it may be desirable for the sequences to be exact matches, i.e. be completely complementary to the sequence to which the oligonucleotide specifically binds, and therefore have zero mismatches along the complementary stretch. As such, highly complementary sequences will typically bind quite specifically to the target sequence region of the Mrna and will therefore be highly efficient in reducing, and/or even inhibiting the translation of the target Mrna sequence into polypeptide product.

[0071] Substantially complementary oligonucleotide sequences will be greater than about 80 percent complementary (or '% exact-match') to the corresponding Mrna target sequence to which the oligonucleotide specifically binds, and will, more preferably be greater than about 85 percent complementary to the corresponding Mrna target sequence to which the oligonucleotide specifically binds. In certain aspects, as described above, it will be desirable to have even more substantially complementary oligonucleotide sequences for use in the practice of the invention, and in such instances, the oligonucleotide sequences will be greater than about 90 percent complementary to the corresponding Mrna target sequence to which the oligonucleotide specifically binds, and may in certain embodiments be greater than about 95 percent complementary to the corresponding Mma target sequence to which the oligonucleotide specifically binds, and even up to and including 96%, 97%, 98%, 99%, and even 100% exact match complementary to all or a portion of the target Mrna to which the designed oligonucleotide specifically binds.

[0072] Percent similarity or percent complementary of any of the disclosed sequences may be determined, for example, by comparing sequence information using the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG). The GAP program utilizes the alignment method of Needleman and Wunsch (1970). Briefly, the GAP program defines similarity as the number of aligned symbols (i.e., nucleotides or amino acids) that are similar, divided by the total number of symbols in the shorter of the two sequences. The preferred default parameters for the GAP program include: (1) a unary

comparison matrix (containing a value of 1 for identities and 0 for non-identities) for nucleotides, and the weighted comparison matrix of Gribskov and Burgess (1986), (2) a penalty of 3.0 for each gap and an additional 0.10 penalty for each symbol in each gap; and (3) no penalty for end gaps.

[0073] 2.6 Exemplary Antisense Constructs for β_1-Adrenoceptor-Specific mRNA

[0074] Table 1 lists representative oligonucleotide sequences contemplated for use in the practice of the present invention.

TABLE 1

EXEMPLARY OLIGONUCLEOTIDE SEQUENCES TARGETED TO MAMMALIAN β_1-AR-ENCODING mRNAs

SEQ ID NO:	SEQUENCE
	Sequences homologous to rat β_1-AR mRNA
SEQ ID NO:1	5'-CCGCGCCCATGCCGA-3'
SEQ ID NO:2	5'-GGCCGACGACAGGTT-3'
SEQ ID NO:3	5'-ATGAGCAGCACGATG-3'
SEQ ID NO:4	5'-**GGGCGCTCGCCCTGGCGCCTCCGAACCCTGCAAC C**-3'
SEQ ID NO:5	5'-**ATGGGCGCGGGGGCGCTCGCCCTGGCGCCTCCGA A**-3'
SEQ ID NO:6	5'-GCCTCCGAATCGGC**ATGGGCGCGGGGGCGCTCGC C**-3'
SEQ ID NO:7	5'-ACCCCCCGCGCCCGGCCTCCGAATCG-GCATGGGCG- 3'
	Sequences homologous to human β_1-AR mRNA
SEQ ID NO:8	5'-**GGGTGCTCGTCCTGGGCGCCTCCGAGCCCGGTAA C**-3'
SEQ ID NO:9	5'-**ATGGGCGCGGGGGTGCTCGTCCTGGGCGCCTCCG A**-3'
SEQ ID NO:10	5'-GCAGCTCGGC**ATGGGCGCGGGGGTGCTCGTCCTG G**-3'
SEQ ID NO:11	5'-CCCGGCCTCCGCAGCTCGGC**ATGGGCGCGGGGGT G**-3'
SEQ ID NO:12	5'-CCGCCCCCGGCCTCCGCAGCTCGGC**ATGGGCGCG G**-3'
SEQ ID NO:13	5'-ACCCCCCGCCCCCGGCCTCCGCAGCTCGGC**ATGG G**-3'
	Sequences homologous to murine β_1-AR mRNA
SEQ ID NO:14	5'-**GGGCGCTCGCCCTGGGCGCCTCCGAACCCTGCAA C**-3'
SEQ ID NO:15	5'-**ATGGGCGCGGGGGCGCTCGCCCTGGGCGCCTCCG A**-3'
SEQ ID NO:16	5'-GCAGCTCGGC**ATGGGCGCGGGGGCGCTCGCCCTG G**-3'
SEQ ID NO:17	5'-CCCGGCCTCCGCAGCTCGGC**ATGGGCGCGGGGGC G**-3'
	Sequences homologous to sheep β_1-AR mRNA
SEQ ID NO:18	5'-**GGGCGCTCGCCCTGGGCGCCTCCGAGCCCTGCAA C**-3'
SEQ ID NO:19	5'-**ATGGGCGCGGGGGCGCTCGCCCTGGGCGCCTCCG A**-3'
SEQ ID NO:20	5'-GCAGCTCGGC**ATGGGCGCGGGGGCGCTCGCCCTG G**-3'
SEQ ID NO:21	5'-CCCGGCCTCCGCAGCTCGGC**ATGGGCGCGGGGGC G**-3'
	Sequences homologous to porcine β_1-AR mRNA
SEQ ID NO:22	5'-**GCGGGGGCGTCGCCCTGGGTGCCTCCGAGCCCTG C**-3'
SEQ ID NO:23	5'-**ATGGGGCGGGGGCGTCGCCCTGGGTGCCTCCGAG C**-3'

TABLE 1-continued

EXEMPLARY OLIGONUCLEOTIDE SEQUENCES TARGETED TO MAMMALIAN β_1-AR-ENCODING mRNAs

SEQ ID NO:	SEQUENCE
SEQ ID NO:24	5'-CGCAGCCGGT**ATGGGGCGGGGGCGTCGCCCTGGG T**-3'
SEQ ID NO:25	5'-CCCCCGCCTCCGCAGCCGGT**ATGGGGCGGGGGCG T**-3'
	Sequences homologous to monkey p_1-AR mRNA
SEQ ID NO:26	5'-**GGGCGCTCGTCCTGGGCGCCTCCGAGCCCGGTAA C**-3'
SEQ ID NO:27	5'-**ATGGGCGCGGGGGCGCTCGTCCTGGGCGCCTCCG A**-3'
SEQ ID NO:28	5'-GCAACTCGGC**ATGGGCGCGGGGGCGCTCGTCCTG G**-3'

N means any nucleotide, e.g., C, A, U (T), or G. Sequences in bold correspond to portions of the β_1-AR open reading frame.

[0075] In addition to the indicated 35-mers, smaller internal n-mers that comprise from at least 9 bases in length up to the full length 35-mers listed are also contemplated to be useful in the practice of the present invention. For example, in addition to the first indicated full-length oligomer, all internal n-mers are also considered to fall within the scope of this disclosure. Thus for each of the 35-mers (SEQ ID NO:4 to SEQ ID NO:28) all internal 34-mers of each sequence as well as all internal 33-mers, 32-mers, 31-mers, 30-mers, 29-mers, 28-mers, 27-mers, 26-mers, 25-mers, 24-mers, 23-mers, 22-mers, 21-mers, 20-mers, 19-mers, 18-mers, 17-mers, 16-mers, 15-mers, 14-mers, 13-mers, 12-mers, 11-mers, 10-mers, and 9-mers, that are comprised within of each of the disclosed 35-mers are also contemplated to be useful in the practice of the present invention.

[0076] For illustrative purposes, all representative n-mers of SEQ ID NO: 10 would include the following internal contiguous 9-mer to 34-mer sequences:

CAGCTCGGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:29)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTCGTCCTG** (SEQ ID NO:30)

AGCTCGGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:31)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTCGTCCT** (SEQ ID NO:32)

GCTCGGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:33)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTCGTCC** (SEQ ID NO:34)

CTCGGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:35)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTCGTC** (SEQ ID NO:36)

TCGGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:37)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTCGT** (SEQ ID NO:38)

CGGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:39)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTCG** (SEQ ID NO:40)

GGC**ATGGGCGCGGGGGTGCTCGTCCTGG** (SEQ ID NO:41)

GCAGCTCGGC**ATGGGCGCGGGGGTGCTC** (SEQ ID NO:42)

-continued

GC**ATGGGCGCGGGGGTGCTCGTCCTGG**	(SEQ ID NO:43)
GCAGCTCGGC**ATGGGCGCGGGGGTGCT**	(SEQ ID NO:44)
C**ATGGGCGCGGGGGTGCTCGTCCTGG**	(SEQ ID NO:45)
GCAGCTCGGC**ATGGGCGCGGGGGTGC**	(SEQ ID NO:46)
ATGGGCGCGGGGGTGCTCGTCCTGG	(SEQ ID NO:47)
GCAGCTCGGC**ATGGGCGCGGGGGTG**	(SEQ ID NO:48)
TGGGCGCGGGGGTGCTCGTCCTGG	(SEQ ID NO:49)
GCAGCTCGGC**ATGGGCGCGGGGGT**	(SEQ ID NO:50)
GGGCGCGGGGGTGCTCGTCCTGG	(SEQ ID NO:51)
GCAGCTCGGC**ATGGGCGCGGGGG**	(SEQ ID NO:52)
GGCGCGGGGGTGCTCGTCCTGG	(SEQ ID NO:53)
GCAGCTCGGC**ATGGGCGCGGGG**	(SEQ ID NO:54)
GCGCGGGGGTGCTCGTCCTGG	(SEQ ID NO:55)
GCAGCTCGGC**ATGGGCGCGGG**	(SEQ ID NO:56)
CGCGGGGGTGCTCGTCCTGG	(SEQ ID NO:57)
GCAGCTCGGC**ATGGGCGCGG**	(SEQ ID NO:58)
GCGGGGGTGCTCGTCCTGG	(SEQ ID NO:59)
GCAGCTCGGC**ATGGGCGCG**	(SEQ ID NO:60)
CGGGGGTGCTCGTCCTGG	(SEQ ID NO:61)
GCAGCTCGGC**ATGGGCGC**	(SEQ ID NO:62)
GGGGGTGCTCGTCCTGG	(SEQ ID NO:63)
GCAGCTCGGC**ATGGGCG**	(SEQ ID NO:64)
GGGGTGCTCGTCCTGG	(SEQ ID NO:65)
GCAGCTCGGC**ATGGGC**	(SEQ ID NO:66)
GGGTGCTCGTCCTGG	(SEQ ID NO:67)
GCAGCTCGGC**ATGGG**	(SEQ ID NO:68)
GGTGCTCGTCCTGG	(SEQ ID NO:69)
GCAGCTCGGC**ATGG**	(SEQ ID NO:70)
GGTGCTCGTCCTGG	(SEQ ID NO:71)
GCAGCTCGGC**ATG**	(SEQ ID NO:72)
GTGCTCGTCCTGG	(SEQ ID NO:73)
GCAGCTCGGC**AT**	(SEQ ID NO:74)
TGCTCGTCCTGG	(SEQ ID NO:75)
GCAGCTCGGC**A**	(SEQ ID NO:76)
GCTCGTCCTGG	(SEQ ID NO:77)
GCAGCTCGGC	(SEQ ID NO:78)
CTCGTCCTGG	(SEQ ID NO:79)
GCAGCTCGG, Etc.	(SEQ ID NO:80)

[0077] In similar fashion, for illustrative purposes, and in the sake of brevity, representative n-mers of SEQ ID NO: 1 would include the following internal contiguous sequences:

CGCGCCCATGCCGA;	(SEQ ID NO:81)
CCGCGCCCATGCCG;	(SEQ ID NO:82)
GCGCCCATGCCGA;	(SEQ ID NO:83)
CCGCGCCCATGCC;	(SEQ ID NO:84)
CGCCCATGCCGA;	(SEQ ID NO:85)
CCGCGCCCATGC;	(SEQ ID NO:86)
GCCCATGCCGA;	(SEQ ID NO:87)
CCGCGCCCATG;	(SEQ ID NO:88)
CCCATGCCGA;	(SEQ ID NO:89)
CCGCGCCCAT;	(SEQ ID NO:90)
CCATGCCGA; and	(SEQ ID NO:91)
CCGCGCCCA.	(SEQ ID NO:92)

[0078] Given the benefit of the present disclosure, the skilled artisan would also be able now, in similar fashion, to prepare any and all possible n-mers from each of the disclosed sequences, and from these sequences, identify and select those particular oligonucleotide sequences that specifically inhibit β_1-AR-specific mRNA expression for therapeutic use by using an acceptable in vitro and/or in vivo assay, such as those described hereinbelow.

[0079] Likewise, based upon the sequence of the human β_1-AR gene, the inventors have identified highly preferred sequences of from about 15 to about 25 nucleotides in length that specifically bind to the mRNA encoding β_1-AR polypeptide, and that reduce the level of polypeptide in a cell expressing such an mRNA. Illustrative sequences in this size range are identified below:

Length = 15 nucleotides:	
5'-CACCCCCGCGCCCAT-3'	(SEQ ID NO:93)
5'-ACCCCCGCGCCCATG-3'	(SEQ ID NO:94)
5'-CGCGCCCATGCCGAG-3'	(SEQ ID NO:95)
5'-GCGCCCATGCCGAGC-3'	(SEQ ID NO:96)
5'-CGCCCATGCCGAGCT-3'	(SEQ ID NO:97)
5'-GCCCATGCCGAGCTG-3'	(SEQ ID NO:98)
5'-CCCATGCCGAGCTGC-3'	(SEQ ID NO:99)
5'-CCATGCCGAGCTGCG-3'	(SEQ ID NO:100)
5'-CATGCCGAGCTGCGG-3'	(SEQ ID NO:101)
5'-ATGCCGAGCTGCGGA-3'	(SEQ ID NO:102)
Length = 16 nucleotides:	
5'-GCGCCCATGCCGAGCT-3'	(SEQ ID NO:103)
5'-CGCCCATGCCGAGCTG-3'	(SEQ ID NO:104)
5'-GCCCATGCCGAGCTGC-3'	(SEQ ID NO:105)

-continued

5'-CCCATGCCGAGCTGCG-3' (SEQ ID NO:106)

5'-CCATGCCGAGCTGCGG-3' (SEQ ID NO:107)

5'-CATGCCGAGCTGCGGA-3' (SEQ ID NO:108)

5'-ATGCCGAGCTGCGGAG-3' (SEQ ID NO:109)

5'-TGCCGAGCTGCGGAGG-3' (SEQ ID NO:110)

Length = 17 nucleotides:

5'-CGCGCCCATGCCGAGCT-3' (SEQ ID NO:111)

5'-GCGCCCATGCCGAGCTG-3' (SEQ ID NO:112)

5'-CGCCCATGCCGAGCTGC-3' (SEQ ID NO:113)

5'-GCCCATGCCGAGCTGCG-3' (SEQ ID NO:114)

5'-CCCATGCCGAGCTGCGG-3' (SEQ ID NO:115)

5'-CCATGCCGAGCTGCGGA-3' (SEQ ID NO:116)

5'-CATGCCGAGCTGCGGAG-3' (SEQ ID NO:117)

5'-ATGCCGAGCTGCGGAGG-3' (SEQ ID NO:118)

Length = 18 nucleotides:

5'-CCGCGCCCATGCCGAGCT-3' (SEQ ID NO:119)

5'-CGCGCCCATGCCGAGCTG-3' (SEQ ID NO:120)

5'-GCGCCCATGCCGAGCTGC-3' (SEQ ID NO:121)

5'-CGCCCATGCCGAGCTGCG-3' (SEQ ID NO:122)

5'-GCCCATGCCGAGCTGCGG-3' (SEQ ID NO:123)

5'-CCCATGCCGAGCTGCGGA-3' (SEQ ID NO:124)

5'-CCATGCCGAGCTGCGGAG-3' (SEQ ID NO:125)

5'-CATGCCGAGCTGCGGAGG-3' (SEQ ID NO:126)

Length = 19 nucleotides:

5'-ACCCCCGCGCCCATGCCGA-3' (SEQ ID NO:127)

5'-CCCGCGCCCATGCCGAGCT-3' (SEQ ID NO:128)

5'-CCGCGCCCATGCCGAGCTG-3' (SEQ ID NO:129)

5'-CGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:130)

5'-GCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:131)

5'-CGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:132)

5'-GCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:133)

5'-CCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:134)

5'-CCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:135)

Length = 20 nucleotides:

5'-CACCCCCGCGCCCATGCCGA-3' (SEQ ID NO:136)

5'-ACCCCCGCGCCCATGCCGAG-3' (SEQ ID NO:137)

5'-CCCCCGCGCCCATGCCGAGC-3' (SEQ ID NO:138)

5'-CCCCGCGCCCATGCCGAGCT-3' (SEQ ID NO:139)

5'-CCCGCGCCCATGCCGAGCTG-3' (SEQ ID NO:140)

5'-CCGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:141)

-continued

5'-CGCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:142)

5'-GCGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:143)

5'-CGCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:144)

5'-GCCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:145)

5'-CCCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:146)

Length = 21 nucleotides:

5'-CACCCCCGCGCCCATGCCGAG-3' (SEQ ID NO:147)

5'-ACCCCCGCGCCCATGCCGAGC-3' (SEQ ID NO:148)

5'-CCCCCGCGCCCATGCCGAGCT-3' (SEQ ID NO:149)

5'-CCCCGCGCCCATGCCGAGCTG-3' (SEQ ID NO:150)

5'-CCCGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:151)

5'-CCGCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:152)

5'-CGCGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:153)

5'-GCGCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:154)

5'-CGCCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:155)

5'-GCCCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:156)

Length = 22 nucleotides:

5'-CACCCCCGCGCCCATGCCGAGC-3' (SEQ ID NO:157)

5'-ACCCCCGCGCCCATGCCGAGCT-3' (SEQ ID NO:158)

5'-CCCCCGCGCCCATGCCGAGCTG-3' (SEQ ID NO:159)

5'-CCCCGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:160)

5'-CCCGCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:161)

5'-CCGCGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:162)

5'-CGCGCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:163)

5'-GCGCCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:164)

5'-CGCCCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:165)

Length = 23 nucleotides:

5'-CACCCCCGCGCCCATGCCGAGCT-3' (SEQ ID NO:166)

5'-ACCCCCGCGCCCATGCCGAGCTG-3' (SEQ ID NO:167)

5'-CCCCCGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:168)

5'-CCCCGCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:169)

5'-CCCGCGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:170)

5'-CCGCGCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:171)

5'-CGCGCCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:172)

5'-GCGCCCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:173)

Length = 24 nucleotides:

5'-CACCCCCGCGCCCATGCCGAGCTG-3' (SEQ ID NO:174)

5'-ACCCCCGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:175)

5'-CCCCCGCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:176)

5'-CCCCGCGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:177)

-continued

5'-CCCGCGCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:178)

5'-CCGCGCCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:179)

5'-CGCGCCCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:180)

Length = 25 nucleotides:

5'-CACCCCCGCGCCCATGCCGAGCTGC-3' (SEQ ID NO:181)

5'-ACCCCCGCGCCCATGCCGAGCTGCG-3' (SEQ ID NO:182)

5'-CCCCCGCGCCCATGCCGAGCTGCGG-3' (SEQ ID NO:183)

5'-CCCCGCGCCCATGCCGAGCTGCGGA-3' (SEQ ID NO:184)

5'-CCCGCGCCCATGCCGAGCTGCGGAG-3' (SEQ ID NO:185)

5'-CCGCGCCCATGCCGAGCTGCGGAGG-3' (SEQ ID NO:186)

3.0 BRIEF DESCRIPTION OF THE DRAWINGS

[0080] The drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0081] **FIG. 1A** shows β_1-AS-ODN decreased density of β_1-ARs but not β_2-ARs in cardiac ventricles of SHRs. A_1 Time Course of β_1-AS-ODN effects on B_{max} of β_1-ARs (●) and β_2-ARs (○).

[0082] **FIG. 1B** shows B_{max} of β-ARs 4 days after intravenous injection of saline (solid bar), 1 mg/kg inverted ODN (open bar), or 1 mg/kg β_1-AS-ODN (shaded bar). Data represent mean±SEM of each point (n=6). *P<0.01, **P<0.001 vs. saline control.

[0083] **FIG. 2**A and **FIG. 2B** show β_1-AS-ODN reduced ISO-stimulated positive inotropic more than chronotropic response in isolated perfused SHR hearts. 48 hr after intravenous injection of 1 mg/kg inverted ODN (○) or β_1-AS-ODN (●), hearts were perfused with Krebs buffer containing increasing concentrations of ISO.

[0084] **FIG. 2A** shows the effect of β_1-AS-ODN on ISO-induced elevation in left ventricular pressure. Data represent mean±SEM of each group (n=6 to 9). *P<0.05, **P<0.01 vs. inverted ODN.

[0085] **FIG. 2B** shows the effect of β_1-AS-ODN on ISO-induced elevation in HR.

[0086] **FIG. 3**A, **FIG. 3**B and **FIG. 3C** show in vivo effects of β_1-AS-ODN and atenolol on cardiovascular hemodynamics of SHRs in response to β_1-stimulation. Same group of SHRs (n=4) was tested for dobutamine-induced hemodynamic alteration at control levels (○), and 1 hr. after atenolol injection (▼). Rats were allowed to fully recover between treatments. During dobutamine infusion,

[0087] **FIG. 3A** shows cardiac dP/dt_{max} of SHRs as monitored by the telemetry system. Data represent mean±SEM. *P<0.01 vs. control.

[0088] **FIG. 3B** shows HR of SHRs as monitored by telemetry system. Data represent mean±SEM. *P<0.01 vs. control.

[0089] **FIG. 3C** shows SBP of SHRs as monitored by telemetry system. Data represent mean±SEM. *P<0.01 vs. control.

[0090] **FIG. 4** shows the effect of β_1-AS-ODN on SBP of SHRs measured with tail cuff. A single dose of 1 mg/kg β_1-AS-ODN was injected into tongue vein with cationic liposome at molar ratio of 1:05 (■) or 1:2.5 (●). Inverted ODN (1 mg/kg) delivered with liposomes at molar ratio of 1:2.5 (○) served as control. Data represent mean±SEM of each group (n=6). *P<0.05 vs. inverted ODN.

[0091] **FIG. 5A** shows the effect of β_1-AS-ODN on mean BP of SHRs monitored by telemetry. Dose of 1 mg/kg inverted ODN (○) or β_1-AS-ODN (●) was injected with liposomes at molar ratio of 1:05. BP and HR were recorded every 10 min. and averaged every 24 hr. Data represent mean±SEM of each group (n=6). *P<0.05 vs. inverted ODN.

[0092] **FIG. 5B** show the effect of β_1-AS-ODN on mean HR of SHRs monitored by telemetry. Dose of 1 mg/kg inverted ODN (○) or β_1-AS-ODN (●) was injected with liposomes at molar ratio of 1:05. BP and HR were recorded every 10 min. and averaged every 24 hr. Data represent mean±SEM of each group (n=6). *P<0.05 vs. inverted ODN.

[0093] **FIG. 6A** shows the effect of atenolol on mean BP of SHRs monitored by telemetry. Saline (○) or 1 mg/kg atenolol (●) was injected intravenously. BP and HR were taken every 10 min. and averaged every 1 hr. Data represent mean±SEM of each group (n=6). *P<0.05 vs. saline.

[0094] **FIG. 6B** shows the effect of atenolol on mean HR of SHRs monitored by telemetry. Saline (○) or 1 mg/kg atenolol (●) was injected intravenously. BP and HR were taken every 10 min. and averaged every 1 hr. Data represent mean±SEM of each group (n=6). *P<0.05 vs. saline.

[0095] **FIG. 7** shows improving the antihypertensive effect of β_1-AS-ODN by optimization of liposome:ODN charge ratios. SHR received a single intravenous injection of β_1-AS-ODN or inverted ODN. β_1-AS-ODN 0.5 mg/kg was delivered by DOTAP/DOPE at charge ratios from 0 to 3.5. Inverted ODN 0.5 mg/kg delivered by DOTAP/DOPE at charge ratio 2.0 served as control. Data represent mean values of each group (n=6). Standard errors were omitted for clarity.

[0096] **FIG. 8**A, **FIG. 8**B and **FIG. 8C** show the effects of β_1-AS-ODN on blood pressure and β-AR levels in renal cortex. SHR were injected with 0.5 mg/kg β_1-AS-ODN or inverted ODN with liposomes at charge ratio 2.

[0097] **FIG. 8A** shows the effect on blood pressure.

[0098] **FIG. 8B** shows the time course of the changes in B_{max} of β_1-AR (●) and β2-AR (○).

[0099] **FIG. 8C** shows the B_{max} of β_1-AR 4 days after intravenous injection of saline (solid bar), inverted ODN (open bar), or β_1-AS-ODN (shaded bar). Data represent mean±SEM of each point (n=6). *P<0.101 vs. saline control.

[0100] **FIG. 9**A, **FIG. 9**B and **FIG. 9C** show β_1-AS-ODN at a single injection exerts a delayed suppression on RAS.

[0101] **FIG. 9A** shows the effect on preprorenin mRNA levels in renal cortex.

[0102] **FIG. 9B** shows the effect on PRA.

[0103] **FIG. 9C** shows the effect on plasma Ang II levels. Data represent mean±SEM of each point (n=6). *P<0.01, **P<0.001 vs. control.

[0104] **FIG. 10** illustrates sustained antihypertensive effects of repeated administrations of β_1-AS-ODN in SHR. Three repeated i.v. injections of 1 mg/kg β_1-AS-ODN (n=9) delivered by liposomes were given to adult SHR as indicated by upward arrows. Repeated injection of saline (n=7) and 1 mg/kg INV-ODN (n=7) served as controls. Systolic BP was measured by tail cuff method. Blood samples were collected at different time points as indicated by downward arrows for safety profile analysis. Data represent mean±SEM. P<0.05 vs. saline or INV-ODN.

[0105] **FIG. 11** illustrates repeated administration of β_1-AS-ODN causes reduced left ventricular hypertrophy in SHR. SHR were treated with repeated injections of β_1-AS-ODN (n=9), saline (n=7) or INV-ODN (n=7) for 2 months. * P<0.

[0106] **FIG. 12** illustrates β_1-AS-ODN and atenolol attenuated ischemia-reperfusion induced cardiac dysfunction. Note the marked increase in LVEDP and CPP and decrease in dLVP in saline-treated rat hearts exposed to a brief ischemia-reperfusion. β_1-AS-ODN, but not INV-ODN, exhibited beneficial effects on cardiac functions, indicated by a smaller increment in CPP and LVEDP and preservation dLVP. Atenolol showed a similar protective effect. Data represent mean±SEM of n=7 for each group.

[0107] **FIG. 13** illustrates plasma levels of liver transaminases ALT and AST after repeated injections of β_1-AS-ODN. Saline and INV-ODN serve as controls. Data represent mean±SEM of each group (n=7-9).

4.0 DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

[0108] 4.1 Antisense Oligonucleotides

[0109] The utility of the disclosed compounds and compositions in inhibiting expression of mammalian β_1-AR-specific mRNA has been described and demonstrated both in vitro and in vivo by the methods described herein.

[0110] Preferred polynucleotide and oligonucleotide compounds of the present invention specifically bind to an mRNA encoding a mammalian β_1-AR polypeptide thereby inhibiting the translation of the mRNA, and concomitant expression of the encoded polypeptide. Highly preferred antisense compounds and compositions are those that specifically bind to the mRNA encoding human β_1-AR polypeptide.

[0111] The mRNA sequence encoding the human β_1-AR polypeptide is disclosed in GenBank™ accession number NM000684, and described herein as SEQ ID NO:187. The mRNA sequence encoding canine β_1-AR polypeptide is disclosed in GenBank™ accession number U73207, and described herein as SEQ ID NO: 188. The mRNA sequence encoding the sheep β_1-AR polypeptide is disclosed in GenBank™ accession numbers S78499 and AF072433, and described herein as SEQ ID NO:189. Likewise, porcine β_1-AR-encoding mRNA is disclosed in GenBank™ accession number AF042454, and described herein as SEQ ID NO:190. Rodent (Rattus) β_1-AR-encoding mRNA is disclosed in GenBank™ accession number D00634 and described herein as SEQ ID NO:191. Rhesus monkey β_1-AR-encoding mRNA is disclosed in GenBank™ accession number X75540, and described herein as SEQ ID NO:192. Murine (Mus) mouse β_1-AR-encoding mRNA is disclosed in GenBank™ accession number L10084, and described herein as SEQ ID NO:193. Highly homologous β_1-AR-encoding DNAs have also been identified in non-mammalian species, such as frog (SEQ ID NO:194) disclosed in GenBank™ as accession number Y09213, further evidence to the fact that antisense oligonucleotides prepared complementary to a β_1-AR-encoding DNA from one species may be useful in reducing the expression of β_1-AR-specific mRNA in another species. In fact, the inventors contemplate that antisense oligonucleotides prepared complementary to highly conserved regions of the proximal portion of the β_1-AR mRNA may specifically bind to, and reduce translation of, the mRNA in a variety of host cells and animals that produce β_1-adrenoceptor polypeptides.

[0112] In the specification and claims, the letters, A, G, C, T, and U respectively indicate nucleotides in which the nucleoside is Adenosine (Ade), Guanosine (Gua), Cytidine (Cyt), Thymidine (Thy), and Uridine (Ura). As used in the specification and claims, compounds that are antisense to the β_1-adrenoceptor-specific PNA, DNA or mRNA sense strand are compounds that have a nucleoside sequence complementary to the sense strand. Table 2 shows the four possible sense strand nucleosides and their complements present in an antisense compound.

TABLE 2

Sense	Antisense
Ade	Thy
Gua	Cyt
Cyt	Gua
Thy	Ade
Ura	Ade

[0113] It will be understood by those skilled in the art that the present invention broadly includes oligonucleotide compounds that are capable of binding to the DNA or mRNA sense strand coding for B_1R. It will also be understood that mRNA includes not only the ribonucleotide sequences encoding a protein, but also regions including the 5'-untranslated region, the 3'-untranslated region, the 5'-cap region and the intron/exon junction regions.

[0114] The invention includes compounds which are not strictly antisense; the compounds of the invention also include those oligonucleotides that may have some bases that are not complementary to bases in the sense strand provided such compounds have sufficient binding affinity for PI-adrenoceptor DNA or mRNA to inhibit expression. In addition, base modifications or the use of universal bases such as inosine in the oligonucleotides of the invention are contemplated within the scope of the subject invention.

[0115] The antisense compounds may have some or all of the phosphates in the nucleotides IS replaced by phosphorothioates (X=S) or methylphosphonates ($X=CH_3$) or other C_{1-4} alkylphosphonates. The antisense compounds optionally may be further differentiated from native DNA by replacing one or both of the free hydroxy groups of the

antisense molecule with C_{1-4} alkoxy groups ($R=C_{1-4}$ alkoxy). As used herein, C_{1-4} alkyl means a branched or unbranched hydrocarbon having 1 to 4 carbon-atoms.

[0116] The disclosed antisense compounds also may be substituted at the 3' and/or 5' ends by a substituted acridine derivative. As used herein, "substituted acridine," means any acridine derivative capable of intercalating nucleotide strands such as DNA. Preferred substituted acridines are 2-methoxy-6-chloro-9-pentylaminoacridine, N-(6-chloro-2-methoxyacridinyl)-O-methoxydiisopropylaminophosphinyl-3-aminopropanol, and N-(6-chloro-2-methoxyacridinyl)-O-methoxydiisopropylaminophosphinyl-5-aminopentanol. Other suitable acridine derivatives are readily apparent to persons skilled in the art. Additionally, as used herein "P(0)(0)-substituted acridine" means a phosphate covalently linked to a substitute acridine.

[0117] As used herein, the term "nucleotides" includes nucleotides in which the phosphate moiety is replaced by phosphorothioate or alkylphosphonate and the nucleotides may be substituted by substituted acridines.

[0118] In one embodiment, the antisense compounds of the invention differ from native DNA by the modification of the phosphodiester backbone to extend the life of the antisense ON. For example, the phosphates can be replaced by phosphorothioates. The ends of the molecule may also be optimally substituted by an acridine derivative that intercalates nucleotide strands of DNA. Intl. Pat. Appl. Publ. No. WO 98/13526 and U.S. Pat. No. 5,849,902 (each specifically incorporated herein by reference) describe a method of preparing three component chimeric antisense compositions, and discuss many of the currently available methodologies for synthesis of substituted oligonucleotides having improved antisense characteristics and/or half-life.

[0119] The reaction scheme involves ^{1}H-tetrazole-catalyzed coupling of phosphoramidites to give phosphate intermediates that are subsequently reacted with sulfur in 2,6-lutidine to generate phosphate compounds. Oligonucleotide compounds are prepared by treating the phosphate compounds with thiophenoxide (1:2:2 thiophenol/triethylamine/tetrahydrofuran, room temperature, 1 hr). The reaction sequence is repeated until an oligonucleotide compound of the desired length has been prepared. The compounds are cleaved from the support by treating with ammonium hydroxide at room temperature for 1 hr and then are further deprotected by heating at about 50° C. overnight to yield preferred antisense compounds.

[0120] The pharmaceutical preparations are made following conventional techniques of a pharmaceutical chemist involving mixing, granulating and compressing, when necessary, for tablet forms or mixing, filling, and dissolving the ingredients, as appropriate, to give the desired oral or parenteral products.

[0121] Doses of the present compounds (in a pharmaceutical dosage unit as described above) will be an efficacious, nontoxic quantity selected from the range of 1 ng/kg to 500 mg/kg of active compound, preferably less than 10 mg/kg. The selected dose is administered to a human patient in need of inhibition of β_1-AR-specific mRNA expression from 1-6 or more times daily, orally, rectally, by injection, or continuously by infusion. Oral formulations would generally require somewhat larger dosages to overcome the effects of gastric decomposition. Intravenous or intra-arterial administration would generally require lower doses since the drug is placed directly into the systemic circulation. Dosages for nasal sprays typically range from about 10 mg to about 50 mg (total) or about 0.1 mg/kg to about 10 mg/kg. Therefore, the dose will depend on the actual route of administration.

[0122] Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence (i.e. in these illustrative examples the rat and human sequences) and determination of secondary structure, T_m, binding energy, relative stability, and antisense compositions were selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell. The results of these analyses are shown in Table 1, for illustrative oligonucleotides and selected target sequences identified in a variety of mammalian and non-mammalian sources.

[0123] Highly preferred target regions of the mRNA, are those that are at or near the AUG translation initiation codon, and those sequences that were substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations were performed using v.4 of the OLIGO primer analysis software (Rychlik, 1997) and the BLASTN 2.0.5 algorithm software (Altschul et al., 1997).

[0124] The sequences of the oligonucleotides given above are highly preferred sequences for inhibiting mammalian β_1-AR polypeptide activity in a host cell. Additional nucleotides at either end derived by the same process (using OLIGO and BLAST) are also envisioned by this invention. The effective lengths of the preferred oligonucleotides are preferably from at least 9 to about 35 or so nucleotides. The inventors contemplate all oligonucleotide compositions in this range (i.e., those of 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 or so bases in length) are highly preferred for the practice of the oligonucleotide-based methods of the invention. In illustrative embodiments, the antisense compounds of the invention differ from native DNA by the modification of the phosphodiester backbone to extend the life of the antisense ODN, in which the phosphate substituents are replaced by phosphorothioates. Likewise, one or both ends of the oligonucleotide may be substituted by one or more acridine derivatives that intercalate between adjacent base-pairs within a strand of nucleic acid.

[0125] 4.2 Methods for Screening Patients at Risk for Hypertension

[0126] Because mammalian β_1-AR polypeptide plays a key role in hypertension in an animal, it is often desirable to measure and quantitate levels of β_1-AR polypeptide in an animal under a variety of conditions, even during the course of a treatment regimen designed to ameliorate the hypertensive condition in such an animal. Likewise, in many instances, it is desirable to employ methods for screening polymorphisms of the gene encoding mammalian β_1-AR, to identify patients "at risk" for hypertension, and to identify alleles of the gene both in vitro and in vivo.

[0127] As such, the use of one or more of the nucleotide compositions described herein as a probe for identifying a gene encoding mammalian β_1-AR, and methods for corre-

lating the presence of such nucleotide segments with the risk of hypertension is particularly desirable.

[0128] 4.3 Co-Administration of Small Molecule and Peptide Inhibitors

[0129] As described herein, in certain embodiments it may be desirable to co-administer one or more of the antisense compositions with one or more pharmaceuticals. For example, one or more of the commercially available anti-hypertensive agents may be co-administered in a particular therapeutic regimen. Such pharmaceuticals include, but are not limited to, amiloride, amlodipine, benazepril, bepridil, candesartan, captopril, chlorothiazide, chlorthalidone, dichlorphenamide, diltiazem, enalapril, ethacrynicacid, felodipine, fosinopril, furosemide, hydrochlorothiazide, hydroflumethiazide, irbesartan, isradipine, lisinopril, losartan, methylclothiazide, metolazone, misoldipine, moexipril, nicardipine, nifedepine, nimodipine, polythiazide, quinapril, ramipril, spironolactone, torsemide, trandolapril, triamterene, valsartan, and verapamil.

[0130] Additional small molecular weight ACE inhibitory compounds and oligopeptides, such as those described in U.S. Pat. No. 5,552,397; U.S. Pat. No. 5,449,661; U.S. Pat. No. 5,348,978; U.S. Pat. No. 5,238,921; U.S. Pat. No. 5,098,887 and U.S. Pat. No. 4,216,209 (each specifically incorporated herein by reference in its entirety). In certain embodiments, such small molecules or other anti-hypertensive agents may also be co-administered to an animal along with one or more of the disclosed antisense constructs.

[0131] The administration of such anti-hypertensive agents or small molecular weight inhibitors is well known to those of skill in the art, and particular, to health practitioners who routinely diagnose and/or treat animals or patients suffering from hypertension.

[0132] 4.4 Pharmaceutical Compositions

[0133] Therefore, in certain embodiments, the present invention also concerns formulation of one or more of the antisense polynucleotide compositions disclosed herein in pharmaceutically acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of antihypertensive therapy.

[0134] It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA antisense compositions disclosed herein may be administered in combination with other agents as well, such as, e.g., proteins or polypeptides or various pharmaceutically-active agents. As long as the composition comprises at least one β_1-AR-specific mRNA inhibitory antisense oligonucleotide, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The RNA, DNA, or PNA-derived antisense compositions may thus be delivered along with various other agents as required in the particular instance. Such RNA, DNA, or PNA antisense compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may comprise substituted or derivatized RNA, DNA, or PNA compositions. Such compositions may include modified peptide or nucleic acid substituent derivatives, as long as the base sequence of the RNA, DNA, or PNA molecule corresponds to one or more of the contiguous base sequences described herein that specifically bind to β_1-AR-specific mRNA, and that reduce or inhibit the extent of translation of this mRNA into biologically-active β_1-AR polypeptides.

[0135] The formulation of pharmaceutically-acceptable excipients and carrier solutions are well-known to those of skill in the art, as is the development of suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including e.g., oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

[0136] 4.4.1 Injectable Delivery

[0137] Alternatively, the pharmaceutical compositions disclosed herein may be administered parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U.S. Pat. No. 5,543,158, U.S. Pat. No. 5,641,515 and U.S. Pat. No. 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free-base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0138] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Pat. No. 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must 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 (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may 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. The prevention of the action of microorganisms can be brought about by various antibacterial ad antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0139] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of

infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and general safety and purity standards as required by FDA Office of Biologics standards.

[0140] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and 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 techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0141] The compositions disclosed herein may be formulated in a neutral or salt form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug release capsules and the like.

[0142] As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

[0143] The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

[0144] 4.4.2 Liposome-, Nanocapsule-, and Microparticle-Mediated Delivery

[0145] In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the antisense compositions of the present invention into suitable host cells. In particular, the antisense oligonucleotide compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

[0146] Such formulations may be preferred for the introduction of pharmaceutically acceptable formulations of the nucleic acids disclosed herein. The formation and use of liposomes is generally known to those of skill in the art (see for example, Couvreur et al., 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-lives (Gabizon and Papahadjopoulos, 1988; Allen and Choun, 1987; U.S. Pat. No. 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran et al., 1997; Margalit, 1995; U.S. Pat. No. 5,567,434; U.S. Pat. No. 5,552,157; U.S. Pat. No. 5,565,213; U.S. Pat. No. 5,738,868 and U.S. Pat. No. 5,795,587, each specifically incorporated herein by reference in its entirety).

[0147] Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC12 cells (Renneisen et al., 1990; Muller et al., 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath and Martin, 1986; Heath et al., 1986; Balazsovits et al., 1989; Fresta and Puglisi, 1996), radiotherapeutic agents (Pikul et al., 1987), enzymes (Imaizumi et al., 1990a; Imaizumi et al., 1990b), viruses (Faller and Baltimore, 1984), transcription factors and allosteric effectors (Nicolau and Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trails examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein et al., 1985a; 1985b; Coune, 1988; Sculier et al., 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori and Fukatsu, 1992).

[0148] Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4 μm. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

[0149] Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, i.e. in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

[0150] In addition to the teachings of Couvreur et al. (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature, and results in an increase in permeability to ions, sugars, and drugs.

[0151] In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins such as cytochrome c bind deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

[0152] The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUWs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

[0153] Alternatively, the invention provides for pharmaceutically acceptable nanocapsule formulations of the polynucleotide compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland et al., 1987; Quintanar-Guerrero et al., 1998; Douglas et al., 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be are easily made, as described (Couvreur et al., 1980; 1988; zur Muhlen et al., 1998; Zambaux et al. 1998; Pinto-Alphandry et al., 1995 and U.S. Pat. No. 5,145,684, specifically incorporated herein by reference in its entirety). In particular, methods of antisense oligonucleotide delivery to a target cell using either nanoparticles or nanospheres (Schwab et al., 1994; Truong-Le et al., 1998) are also particularly contemplated to be useful in formulating the disclosed compositions for administration to an animal, and to a human in particular.

[0154] 4.5 Therapeutic and Diagnostic Kits

[0155] The invention also encompasses one or more of the antisense compounds together with one or more pharmaceutically-acceptable excipients, carriers, diluents, adjuvants, and/or other components, such as additional antihypertensive agents, oligonucleotides, peptides, antigens, or other therapeutic compounds as may be employed in the formulation of particular oligonucleotide or polynucleotide delivery formulations, and in the preparation of antihypertensive agents or cardiac therapy for administration to an animal.

[0156] As such, preferred animals for administration of the pharmaceutical compositions disclosed herein include mammals, and particularly humans. Other preferred animals include primates, sheep, goats, bovines, equines, porcines, lupines, canines, and felines, as well as any other mammalian species commonly considered pets, livestock, or commercially relevant species. The composition may include partially or significantly purified antisense compositions, either alone, or in combination with one or more additional active ingredients, which may be obtained from natural or recombinant sources, or which may be obtainable naturally or either chemically synthesized, or alternatively produced in vitro from recombinant host cells expressing nucleic acid segments encoding such additional active ingredients.

[0157] Therapeutic kits may also be prepared that comprise at least one of the β_1-AR mRNA-specific antisense oligonucleotides and polynucleotides disclosed herein and instructions for using the composition as a therapeutic agent. The container means for such kits may typically comprise at least one vial, test tube, flask, bottle, syringe or other container means, into which the antisense composition(s) may be placed, and preferably suitably aliquoted. Where a second antihypertensive agent is also provided, the kit may also contain a second distinct container means into which this second composition may be placed. Alternatively, the plurality of antihypertensive compositions may be prepared in a single pharmaceutical composition, and may be packaged in a single container means, such as a vial, flask, syringe, bottle, or other suitable single container means. The kits of the present invention will also typically include a means for containing the vial(s) in close confinement for commercial sale, such as, e.g., injection or blow-molded plastic containers into which the desired vial(s) are retained.

[0158] Alternatively, for the preparation of diagnostic kits, and for methods relating to the use of these compounds in the identification of β_1-AR polypeptide-encoding nucleic acids in a biological sample, such kits may be prepared that comprise at least one of the β_1-adrenoceptor mRNA-specific antisense oligonucleotides disclosed herein and instructions for using the composition as a probe for β_1-AR-specific nucleic acids in a hybridization assay. The container means for such kits may typically comprise at least one vial, test tube, microcentrifuge tube, or other container means, into which the antisense composition(s) may be placed and suitably aliquoted. Where a radiolabel or fluorigenic label or other such detecting means is included within the kit, the labeling agent may be provided either in the same container as the oligonucleotide composition, or may alternatively be placed in a second distinct container means into which this second composition may be placed and suitably aliquoted. Alternatively, the oligonucleotide composition and the label may be prepared in a single container means, and in most cases, the kit will also typically include a means for containing the vial(s) in close confinement for commercial sale and/or convenient packaging and delivery.

[0159] 4.6 Peptide Nucleic Acid Compositions

[0160] In certain embodiments, the inventors contemplate the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the

nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, 1997). PNAs may be utilized in a number of methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. An excellent review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (1997) and is incorporated herein by reference. As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the β_1-adrenoceptor-specific mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of β_1-adrenoceptor-specific mRNA, and thereby alter the level of β_1-adrenoceptor polypeptide in a host cell to which such PNA compositions have been administered.

[0161] PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen et al., 1993; Hanvey et al., 1992; Hyrup and Nielsen, 1996; Nielsen, 1995). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc (Dueholm et al., 1992) or Fmoc (Bonham et al., 1995) protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used (Christensen et al., 1995).

[0162] PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, Mass., USA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton et al., 1995).

[0163] 4.7 Methods of Nucleic Acid Delivery and DNA Transfection

[0164] In certain embodiments, it is contemplated that one or more RNA, DNA, PNAs and/or substituted polynucleotide compositions disclosed herein will be used to transfect an appropriate host cell. Technology for introduction of PNAs, RNAs, and DNAs into cells is well known to those of skill in the art.

[0165] Several non-viral methods for the transfer of expression constructs into cultured mammalian cells also are contemplated by the present invention. These include calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe et al., 1990) DEAE-dextran (Gopal, 1985), electroporation (Wong and Neumann, 1982; Fromm et al., 1985; Tur-Kaspa et al., 1986; Potter et al., 1984; Suzuki et al., 1998; Vanbever et al., 1998), direct microinjection (Capecchi, 1980; Harland and Weintraub, 1985), DNA-loaded liposomes (Nicolau and Sene, 1982; Fraley et al., 1979; Takakura, 1998) and lipofectamine-DNA complexes, cell sonication (Fechheimer et al., 1987), gene bombardment using high velocity microprojectiles (Yang et al., 1990; Klein et al., 1992), and receptor-mediated transfection (Curiel et al., 1991; Wagner et al., 1992; Wu and Wu, 1987; Wu and Wu, 1988). Some of these techniques may be successfully adapted for in vivo or ex vivo use.

[0166] Moreover, the use of viral vectors (Lu et al., 1993; Eglitis and Anderson, 1988; Eglitis et al., 1988), including retroviruses, baculoviruses, adenoviruses, adenoassociated viruses, vaccinia viruses, Herpes viruses, and the like are well-known in the art, and are described in detail herein.

[0167] 4.8 Expression Vectors

[0168] The present invention contemplates an expression vector comprising at least one β_1-AR-specific polynucleotide of the present invention. Thus, in one embodiment an expression vector is constructed with a specific DNA molecule orientated in the antisense direction. In another embodiment, a promoter is operatively linked to a sequence region that encodes a functional RNA such as a tRNA, a ribozyme or an antisense RNA.

[0169] As used herein, the term "operatively linked" means that a promoter is connected to a functional RNA in such a way that the transcription of that functional RNA is controlled and regulated by that promoter. Means for operatively linking a promoter to a functional RNA are well known in the art.

[0170] The choice of which expression vector and ultimately to which promoter a polypeptide coding region is operatively linked depend directly on the functional properties desired, e.g., the location and timing of protein expression, and the host cell to be transformed. These are well known limitations inherent in the art of constructing recombinant DNA molecules. However, a vector useful in practicing the present invention is capable of directing the expression of the functional RNA to which it is operatively linked.

[0171] RNA polymerase transcribes a coding DNA sequence through a site where polyadenylation occurs. Typically, DNA sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate transcription. Those DNA sequences are referred to herein as transcription-termination regions. Those regions are required for efficient polyadenylation of transcribed messenger RNA (mRNA).

[0172] A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini or blunt ends. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

[0173] 4.9 In vivo Delivery and Treatment Protocols

[0174] To introduce the antisense constructs to cells in vivo, one of any number of conventional ways may be employed. These methods include viral-mediated delivery using retroviral, adenoviral, or adeno-associated viral vectors, and are well known to those of skill in the antisense therapeutic arts.

[0175] Adenovirus vectors have been used in eukaryotic gene expression (Levrero et al., 1991; Gomez-Foix et al., 1992) and vaccine development (Grunhaus and Horwitz, 1992; Graham and Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet and Perricaudet, 1991; Stratford-Perricaudet et al., 1990; Rich et al., 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld et al., 1991; Rosen-

feld et al., 1992), muscle injection (Ragot et al., 1993), peripheral intravenous injections (Herz and Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle et al., 1993).

[0176] The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5' and 3' ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

[0177] AAV (Ridgeway, 1988; Hermonat and Muzycska, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies are present in 85% of the U.S. human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replication is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of 20 to 24 nm in diameter (Muzyczka and McLaughlin, 1988).

[0178] The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs (**FIGS. 2A, 2B** and **2**C). There are two major genes in the AAV genome: rep and cap. The rep gene encodes proteins responsible for viral replications, whereas the cap gene encodes the capsid protein VP 1-3. Each ITR forms a T-shaped hairpin structure. These terminal repeats are the only essential cis components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of rep proteins, and transcription from p40 produces the capsid proteins (Hermonat and Muzyczka, 1984).

[0179] Other viral vectors may also be employed as expression constructs in the present invention for the delivery of β_1-AR mRNA-complementary oligonucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar et al., 1988), lentiviruses, polioviruses and herpesviruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar et al., 1988; Horwich et al., 1990).

[0180] In order to effect expression of the β_1-adrenoceptor mRNA-complementary antisense sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished in vitro, as in laboratory procedures for transforming cells lines, or in vivo or ex vivo, as in the treatment of certain disease states, and in particular, in the treatment of hypertension and β_1-AR-related disorders. As described above, the preferred mechanism for delivery is via viral infection where the expression construct is encapsidated in an infectious viral particle.

[0181] Once the expression construct has been delivered into the cell the nucleic acid encoding the β_1-adrenoceptor mRNA-complementary oligonucleotide antisense sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the antisense construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation via homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

[0182] In one embodiment of the invention, the expression construct comprising one or more β_1-AR mRNA-complementary oligonucleotide or polynucleotide antisense sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer in vitro but it may be applied to in vivo use as well. Dubensky et al. (1984) successfully injected polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes. It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner in vivo and express the gene product.

[0183] Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity allowing them to pierce cell membranes and enter cells without killing them (Klein et al., 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang et al., 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

[0184] Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded in vivo (Yang et al., 1990; Zelenin et al., 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, i.e. ex vivo treatment. Again, DNA encoding a particular gene may be delivered via this method and still be incorporated by the present invention.

[0185] In a further embodiment of the invention, the expression construct comprising an β_1-AR mRNA-comple-

mentary oligonucleotide antisense sequence may be entrapped in one or more nanocapsules, liposomes, or other lipid based DNA delivery agent. Liposomes are vesicular structures characterized by a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures, and entrap water and dissolved solutes between the lipid bilayers (Ghosh and Bachhawat, 1991). Also contemplated are lipofectamine-DNA complexes.

[0186] Liposome-mediated nucleic acid delivery and expression of foreign DNA in vitro has been very successful. Wong et al. (1980) demonstrated the feasibility of liposome-mediated delivery and expression of foreign DNA in cultured chick embryo, HeLa and hepatoma cells. Nicolau et al. (1987) accomplished successful liposome-mediated gene transfer in rats after intravenous injection.

[0187] In certain embodiments of the invention, the liposome may be complexed with a hemagglutinating virus (HVJ). This has been shown to facilitate fusion with the cell membrane and promote cell entry of liposome-encapsulated DNA (Kaneda et al., 1989). In other embodiments, the liposome may be complexed or employed in conjunction with nuclear non-histone chromosomal proteins (HMG-1) (Kato et al., 1991). In yet further embodiments, the liposome may be complexed or employed in conjunction with both HVJ and HMG-1. In that such expression constructs have been successfully employed in transfer and expression of nucleic acid in vitro and in vivo, then they are applicable for the present invention. Where a bacterial promoter is employed in the DNA construct, it also will be desirable to include within the liposome an appropriate bacterial polymerase.

[0188] Other expression constructs that may be employed to deliver an antisense polynucleotide into a target cell include receptor-mediated delivery vehicles. These take advantage of the selective uptake of macromolecules by receptor-mediated endocytosis in almost all eukaryotic cells. Because of the cell type-specific distribution of various receptors, the delivery can be highly specific (Wu and Wu, 1993).

[0189] Receptor-mediated gene targeting vehicles generally consist of two components: a cell receptor-specific ligand and a DNA-binding agent. Several ligands have been used for receptor-mediated gene transfer. The most extensively characterized ligands are asialoorosomucoid (ASOR) (Wu and Wu, 1987) and transferrin (Wagner et al., 1993). Recently, a synthetic neoglycoprotein, which recognizes the same receptor as ASOR, has been used as a gene delivery vehicle (Ferkol et al., 1993; Perales et al., 1994) and epidermal growth factor (EGF) has also been used to deliver genes to squamous carcinoma cells (Eur. Pat. Appl. Publ. No. EP0273085, specifically incorporated herein by reference in its entirety).

[0190] In other embodiments, the delivery vehicle may comprise a ligand and a liposome. For example, Nicolau et al. (1987) employed lactosyl-ceramide, a galactose-terminal asialganglioside, incorporated into liposomes and observed an increase in the uptake of the insulin gene by hepatocytes. Thus, it is feasible that a nucleic acid encoding a particular gene also may be specifically delivered into a cell type such as lung, epithelial or tumor cells, by any number of receptor-ligand systems with or without liposomes. For example, epidermal growth factor (EGF) may be used as the receptor for mediated delivery of a nucleic acid encoding a gene in many tumor cells that exhibit upregulation of EGF receptor. Mannose can be used to target the mannose receptor on liver cells. Also, antibodies to CD5 (CLL), CD22 (lymphoma), CD25 (T-cell leukemia) and MAA (melanoma) can similarly be used as targeting moieties.

[0191] In certain embodiments, gene transfer may more easily be performed under ex vivo conditions. Ex vivo gene therapy refers to the isolation of cells from an animal, the delivery of a nucleic acid into the cells in vitro, and then the return of the modified cells back into an animal. This may involve the surgical removal of tissue/organs from an animal or the primary culture of cells and tissues. Anderson et al., U.S. Pat. No. 5,399,346, and incorporated herein in its entirety, disclose ex vivo therapeutic methods.

5.0 EXAMPLES

[0192] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

5.1 Example 1

Antisense Inhibition of β-AR mRNA

[0193] β_1-antisense, through specific inhibitions of β_1-AR expression, decreases the functional sensitivity of β_1-AR-mediated responses in the face of sympathetic activation and thereby achieves an antihypertensive effect. In this example, an AS-ODN was designed complementary to rat β_1-AR mRNA and its ability was demonstrated to inhibit β_1-AR density and function in the heart and to reduce BP in spontaneously hypertensive rats.

[0194] 5.1.1 Methods

[0195] 5.1.1.1 Antisense Design and Administration

[0196] AS-ODN and inverted ODN control were 15-mer and targeted to the AUG start codon of rat β_1-AR mRNA (Machida et al., 1990). The sequence of AS-ODN is 5'-CCGCGCCCATGCCGA-3' (SEQ ID NO:195), and the inverted ODN is 5'-AGCCGTACCCGCGCC-3' (SEQ ID NO:196). This AS-ODN was chosen from 6 AS candidates targeted to different regions of β_1-AR mRNA on the basis of the intensity of cardiac β_1-AR inhibition and reduction of BP in SHRs. These oligonucleotides were modified by backbone phosphorothioation. ODNs delivered with cationic liposomes were injected into the tongue vein.

[0197] 5.1.1.2 Preparation of Liposomes and ODN/Liposome Complex

[0198] The cationic lipid 1,2-bis(oleoyloxy)-3-(trimethylammonio)propane (DOTAP) was mixed with helper lipid 1-α-dioleoyl phosphatidylethanolamine (DOPE, Avanti Polar Lipids, Alabaster, Ala.) at a 1:1 molar ratio, briefly sonicated, and stored at 4° C. until use. The average diameter of liposomes is 200 to 300 nm (Tang and Hughes, 1998). ODN/liposome complex was prepared on the day of use by mixing the desired amounts of ODNs with DOTAP/DOPE to the final DNA concentration of 300 μg/mL in 5% wt./vol. dextrose in water and incubating at room temperature for 60 min. Two DNA/lipid molar ratios, i.e. 1:0.5 and 1:2.5, were used in the studies.

[0199] 5.1.1.3 Animal Surgery

[0200] Adult male SHRs (250 to 350 g, Harlan, Indianapolis, Ind.) were kept in cages in a room with a 12-hr. light-dark cycle. Animals were fed standard laboratory rat chow and tap water ad libitum.

[0201] 5.1.1.4 Telemetric Sensor Implantation

[0202] Before implantation, the zero of each radiotransmitter (TA11PA-C40, Data Sciences, St. Paul, Minn.) was verified to be<4 mm Hg. SHRs were anesthetized with 100 mg/kg ketamine and 15 mg/kg xylazine, and a midline abdominal incision was made. A fluid-filled sensor catheter was then inserted into the right femoral artery, and the tip of the catheter was in the abdominal aorta caudal to the renal arteries. The rats with implants were allowed to recover for 1 week.

[0203] 5.1.1.5 Jugular Vein Cannulation

[0204] One week after telemetric implantation, rats were anesthetized, and a curved catheter made of PE 50 and vinyl tubing was inserted into a curved catheter made of PE 50 and vinyl tubing was inserted into the jugular vein. The tubing was led under the skin of the neck and exposed on the back to allow for drug infusion. Rats were allowed to recovery for 24 hr before experimentation. The catheters were flushed with 100 U heparin every day to prevent clogging.

[0205] 5.1.1.6 Membrane Preparation and β-AR Binding Assay

[0206] Four days after intravenous injection of saline (n=6) or 1 mg/kg inverted ODN (n=6) or 2, 4, 10 or 18 days after injection of 1 mg/kg β_1-AS-ODN (n=24), animals were euthanized, and membranes were prepared from heart ventricles as previously described (Baker and Pitha, 1982). For saturation experiments, 100 μg membrane protein was incubated in triplicate with 6 concentrations of [^{125}I](⁻)-iodocyanopindolol (ICYP, NEN Life Science, 6.25 to 100 pmol/L) in a total volume of 250 μL containing 50 mmol/L Tris-HCl (pH 7.4), 5 mmol/L $MgCl_2$ at 36° C. for 60 minutes. The nonspecific and β_2-AR-binding levels were determined in the presence of 1 μmol/L (±)-alprenolol and 150 mmol/L CGP20712A (RBI), respectively. Then the reaction mixture was passed through Whatman (GF/B glass fiber filter using a Brandel harvester, and the bound radioactivity was counted for 1 min.

[0207] 5.1.1.7 Tissue Preparation and Quantitative Autoradiography

[0208] Four days after injection of 1 mg/kg β_1-AS-ODN (n=6) or saline (n=6), rats were killed, and tissues were removed and frozen in dry ice. Coronal sections of brain, horizontal sections of heart, and sagittal sections of kidney (20 μm) were cut on a cryostat (Microm, Thornwood, N.Y.) at ⁻20° C. and mounted on microscope slides. Every seventh slide was stained with hematoxylin and eosin for histology. Tissue sections were preincubated in Krebs buffer (mmol/L: NaCl 118.4, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 1.27 and NaH_2PO_4 10.0, pH 7.1) containing 0.1 mmol/L GTP, 0.1 mmol/L ascorbic acid, and 10 μmol/L PMSF for 30 minutes at 25° C. Sections were then incubated in Krebs buffer containing 0.1 mmol/L ascorbic acid and 10 μmol/L PMSF with 100 pmol/L ICYP at 25° C. for 150 minutes in the presence of 1 μmol/L (⁻) propranolol, 100 mmol/L ICI 118,551 (β_1-selective antagonist), or 100 mmol/L CGP20712A (β_1-selective antagonist) to distinguish nonspecific, β_1-, and β_2-bindings. Labeled sections were rinsed in the same buffer, followed by two 15-min. washes at 37° C. in the buffer, and rinsed in distilled water at 25° C. (Matthews et al., 1994). Dried sections were then exposed to x-ray films. The images were quantified with a computerized image analysis system (MCID, Imaging Research) and normalized with ^{125}I standards. Nonspecific binding was <10% of total binding.

[0209] 5.1.1.8 Determination of Effects of β_1-AS and Atenolol on Cardiovascular Parameters in Response to β-Stimulation

[0210] 48 hr after injection of 1 mg/kg β_1-AS-ODN (n=9) or inverted ODN (n=6), SHRs were anesthetized and killed. Hearts were quickly removed and perfused via the aorta with oxygenated Krebs buffer (118 mmol/L NaCl, 18.75 mmol/L $NaHCO_3$ 1.2 mmol/L KH_2PO_4, 4.7 mmol/L KCl, 1.2 mmol/L $MgSO_4$, 1.25 mmol/L $CaCl_2$, 11.1 mmol/L glucose, and 0.01 mmol/L EDTA) at a constant flow of 7.0 mL/min. at 36° C. Coronary perfusion pressure was measured via a catheter placed proximal to the aorta and connected to a pressure transducer (Gould Staham P231D, Eastlake, Ohio). A latex balloon filled with water and connected to the pressure transducer was inserted into the left ventricle through the left atrium to measure left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), and developed left ventricular pressure (dLVP) (dLVP=LVSP-LVEDP). LVEDP during equilibration was set at 5 to 7 mm Hg. Coronary perfusion pressure, LVEDP, and LVSP were recorded continuously on a 4-channel recorder (Astro-Med, West Warwick, R1). After baseline values for dLVP and heart rate (HR) were stable for 5 min., isoproterenol (ISO, nonspecific β-agonist) was given at 0.01, 0.025, 0.05, and 0.12 μmol/L at 10 min. intervals so as to avoid the effect of tachyphylaxis.

[0211] The effects of β_1-AS-ODN and atenolol on cardiac dP/dt_{max}, and systolic blood pressure (SBP) were compared in the same group of SHRs (n=4). Two days after catherization of the jugular vein, control values were taken and 1 mg/kg β_1-AS-ODN was injected. 48 hr later, rats were tested for the effect of β_1-AS-ODN. The rats were allowed to recover until all the cardiovascular parameters returned to control values. Then 1 mg/kg atenolol (β_1-selective antagonist) was injected, and rats were tested 30 min. later. For

β_1-stimulation, SHRs were infused with dobutamine (β_1-selective agonist) through a jugular vein catheter at 5, 10, 20, and 40 $\mu g \cdot kg^{-1} \cdot min.^{-1}$. Each dose was given for 5 min. continuously and at 1-hr intervals until all the cardiovascular parameters returned to baseline values so as to avoid the effect of tachyphylaxis. BP and HR were sampled every 1 min. dP/dt_{max} was calculated from the slope of the rising pulse-pressure curve and determined every 1 min. The difference between values at each dose and baseline was denoted as A.

[0212] 5.1.1.9 BP Monitoring

[0213] Each rat cage was placed on a receiver (RLA 1020, Data Sciences, St. Paul, Minn.) for measurement of cardiovascular parameters. Data were collected with a computer-based data acquisition program (Dataquest Lab-PRO3.0; Data Sciences). BP and HR were measured every 10 min. and averaged every 1 to 24 hrs. Before treatment, SHRs were monitored for a week to get a stable baseline.

[0214] Rats were warmed for 20 to min. in cages on heating pads. The temperature was controlled at 35° C. to 37° C. Then the rats were placed in a plastic restrainer kept at 37° C. A pneumatic pulse sensor was attached to the tail. After cuff inflation, SBP was determined as the first pulsatile oscillation on the descending side of the pressure curve. HR was determined by manual counting of pulse numbers per unit time. BP and HR were recorded by a Narco physiograph. Data values of each rat were taken as an average of at least 4 stable readings. Baseline was determined by averaging 3 days of measurements before antisense administration.

[0215] 5.1.1.10 Statistical Analysis

[0216] Values were expressed as mean±SEM. The difference was considered statistically significant at $P<0.05$. An unpaired t test was used to compare B_{max}, dLVP, and BP in 2 groups. One-way repeated-measures ANOVA and Tukey test were used to compare $\Delta dP/dt$ and ΔHR on dobutamine infusion in different groups. Pearson product-moment correlation was used to assess the relationship between β_1-AR B_{max} and dLVP.

[0217] 5.1.2 Results

[0218] 5.1.2.1 Effect on Cardiac β-AR Density

[0219] **FIG. 1A** shows that a single intravenous injection of 1 mg/kg β_1-AS-ODN delivered with cationic liposomes at a 1:2.5 molar ratio significantly reduced the β_1-AR density in the SHR hearts for 18 days ($P<0.01$). The drop was at its maximum of 47% on day 4, 33% on day 10 and maintained at 29% on day 18. In contrast, there was no significant change in the B_{max} of β_2-ARs. The K_D of both subtypes remained unaltered (Table 1). Consequently, the β_1/β_2 subtype ratio in the ventricles was diminished from ≈70/30 to ≈50/50 by β_1-AS-ODN. Inverted ODN had no effect on either subtype (**FIG. 1B**).

[0220] 5.1.2.2 Effect on Cardiac Contractility and HR in Response to β-Stimulation

[0221] 48 hr after injection of 1 mg/kg β_1-AS-ODN, the cardiac inotropic and chronotropic responses to β-stimulation were determined in SHRs in vitro and in vivo.

[0222] First, isolated hearts were perfused with incrementing doses of ISO, which enhanced HR and contractility via activating β-ARs. The dLVP-ISO dose-response curve, which reflected the positive inotropic effect of ISO, was significantly shifted downward by β_1-AS-ODN ($P<0.02$). HR was not significantly decreased, except at 1 point, i.e. 0.01 μmol/L ISO ($P<0.05$) (**FIG. 2A** and **FIG. 2B**).

[0223] An in vivo test was performed in conscious SHRs monitored by radiotelemetry. β_1-AS-ODN significantly ($P<0.02$) dampened the increase in dP/dt_{max} in the face of dobutamine (β_1-selecrive agonist) (**FIG. 3A**). The change in HR was not significantly reduced (**FIG. 3B**), which echoed the results in isolated perfused hearts. Conversely, the response of BP to dobutamine was biphasic (**FIG. 3C**). Dobutamine elevated SBP by 5 to 8 mm Hg at low infusion speed and reduced SBP at higher speed, probably as a result of the partial β_2-agonistic activity of dobutamine at high doses and the consequent vasodilatory effect on BP. **FIG. 3** also compares the results with β_1-AS-ODN and atenolol (β_1-selective antagonist). Relative to β_1-AS-ODN, 1 mg/kg atenolol produced a more profound decline in $\Delta dP/dt_{max}$ and ΔHR during a 5 hr. period of time after injection. Moreover, it reduced basal dP/dt_{max} (2450±295 mm Hg/s versus control, 2937±277 mm Hg/s) and caused bradycardia (295±12 bpm versus control, 365±8 bpm) ($P<0.05$), whereas β_1-AS did not change basal contractility (2922±249 mm Hg/s) or HR (365±12 bpm). But the effects of atenolol on $\Delta dP/dt_{max}$ and ΔHR were transient. Within 24 hr after atenolol administration, the inotropic and chronotropic effects of dobutamine had returned to control levels.

TABLE 3

B_{MAX} AND K_D OF β_1- AND β_2-ARS IN CARDIAC VENTRICLES OF SHRS 4 DAYS AFTER TREATMENT WITH SALINE, INVERTED ODN, OR β_1-AS-ODN

	Total (β_1+β_2)		β_1		β_2	
	K_D, pmol/L	B_{max}, fmol/mg	K_D, pmol/L	B_{max}, fmol/mg	K_D, pmol/L	B_{max}, fmol/mg
Saline	55.4 ± 6.7	27.6 ± 0.9	64.7 ± 5.4	18.7 ± 0.9	29.6 ± 0.5	9.1 ± 0.6
Inverted ODN	57.1 ± 3.5	26.3 ± 3.0	66.0 ± 1.0	18.1 ± 2.3	32.0 ± 2.4	8.7 ± 0.5
β_1-AS-ODN	37.0 ± 1.0*	19.8 ± 1.8*	60.5 ± 3.4	10.0 ± 1.2*	30.7 ± 3.3	9.9 ± 0.5

Data represent mean ± SEM of each group (n = 6 to 10)
*$p < 0.01$ vs. saline control

[0224] 5.1.2.3 Effect on BP of SHRs

[0225] **FIG. 4** shows the effects of a single injection of 1 mg/kg β_1-AS-ODN on the BP of SHRs measured by the tail-cuff method. AS-ODN was delivered with cationic liposomes at different molar ratios of DNA/lipid, i.e. 1:0.5 and 1:2.5. β_1-AS-ODN delivered with liposomes at a 1:0.5 ratio diminished SBP for 8 days. The maximum drop was 38±5 mm Hg. When the molar ratio was increased to 1:2.5, this hypotensive effect was drastically prolonged to 20 days. No effect was seen with inverted ODN.

[0226] To compare the effects of β_1-AS-ODN and atenolol, radiotelemetry was used to monitor BP and HR on a regular basis. β_1-AS-ODN 1 mg/kg delivered with liposomes at a 1:0.5 ratio produced a maximum drop of 15 mm Hg in mean BP (**FIG. 5A**). The antihypertensive effect lasted for 8 days, which was consistent with the results measured with the tail cuff. HR was not significantly altered (**FIG. 5B**). In contrast to AS-ODN, although the onset of the hypotensive effect caused by 1 mg/kg atenolol occurred as early as 20 min. after injection, it lasted for only 10 hr (**FIG. 6A**). In addition, atenolol caused considerable bradycardia up to an average of ~75 bpm (**FIG. 6B**).

[0227] 5.1.2.4 Effect on β-AR Distribution in Brain, Heart, and Kidney

[0228] Quantitative autoradiography of 6 to 18 tissue slices in brain, heart, and kidney was analyzed 4 days after intravenous β_1-AS-ODN administration. No changes in the distribution of β-ARs in the forebrain and brain stem regions were detected. This indicated the absence of antisense effect on the β-AR expression in CNS. However, β_1-AS-ODN significantly ($P<0.05$) reduced β_1-AR density in cardiac ventricles (from 30.2±2.1 to 20.6±2.5 fmol/mg) and renal cortex (from 26.4±3.1 to 17.4±3.3 fmol/mg). This was consistent with the binding results. β_2-ARs were not affected in any tissues.

[0229] 5.1.3 Discussion

[0230] This example compares the effects of a novel β_1-AS-ODN on high BP in a model of hypertension with a currently used β-blocker. β_1-AS-ODN knocked down β_1-adrenergic activity, resulting in long-term attenuation of BP. The results indicated that β_1-AS-ODN reduced β_1-AR density and cardiac contractility after β_1-stimulation in vitro and in vivo and lowered high BP of SHRs. These findings are consistent with the hypothesis that β_1-AS, through the inhibition of β_1-AR expression, is able to render heart, kidney, and other tissues less sensitive to sympathetic activation, which is a major contributing factor in high BP. A single injection of β_1-AS-ODN effectively decreased the cardiac β_1-AR density, which was accompanied by diminished ventricular contractility and cardiac output in response to β-stimulation. The antihypertensive effect in SHRs was up to a 38 mm Hg reduction lasting as long as 20 days.

[0231] Treatment of β_1-AS-ODN reduced cardiac β_1-AR density by ≈50% in 4 days. Considerable variation is reported in the literature on the half-life of β_1-ARs (Baker and Pitha, 1982; Neve and Molinoff, 1986 and Winter et al., 1988). From the results with the AS-ODN inhibition, the half-life of cardiac β_1-ARs is ≈2 to 4 days to allow for 50% reduction of β-AR density within 4 days.

[0232] Both tail-cuff and telemetry measures of BP showed a significant drop after antisense treatment. SHRs responded to β_1-AS-ODN to a greater degree of hypotension when subjected to tail cuff vs. telemetry. In addition, the baseline measured with the tail cuff was consistently higher than that with telemetry by 20 to 30 mm Hg in the same rats. Bazil et al., 1993 reported a similar phenomenon. They compared the cardiovascular parameters recorded by telemetry, tail cuff, and arterial catheter and observed a more sensitive hypotensive effect of captopril with tail cuff. Tail-cuff measurement of BP involves warming and restraint of rats. It is conceivable that β_1-AS, through the suppression of sympathetic activity, can decrease the BP of animals under stress more effectively.

[0233] Cationic liposomes are effective vehicles for gene delivery. It has been shown that liposomes entrapment not only improves the cellular uptake of DNA but also protects DNA from degradation and extends its circulation time (Allen, 1997; Liu et al., 1997). Numerous factors influence the efficiency of cationic liposome-mediated intravenous gene delivery, such as DNA/lipid ratio, selection of lipids, and preparation procedure. A widely used lipid formula, DOTAP/DOPE, was used to deliver β_1-AS-ODN at 4 molar ratios. The optimal ratio of 1:2.5 was determined on the basis of the duration and magnitude of the antihypertensive effects. A profound and prolonged fall in BP of 38 mm Hg up to 20 days was achieved at this ratio, which followed the reduction in β_1-AR binding to a maximum of 47% at day 4, 33% at day 10, and 29% at day 18. This implies that β_1-AS-ODN effectively inhibits the functionally active receptors involved in the BP.

[0234] The blood-brain barrier formed by capillary endothelia is permeable only to small lipophilic molecules with a molecular weight of <600 Da (reviewed by Pardridge, 1998). Owing to their high hydrophilicity, ODNs undergo negligible transport through blood-brain barrier and have very limited access to CNS. The cellular and organ distributions of DNA/liposome complexes with fluorescent labeling were previously studied in mice after intravenous injection, and the results indicated that the complexes were taken up primarily by capillary endothelial cells in most of the peripheral organs, including lung, heart, kidney, and spleen, but were absent in the brain (McLean et al., 1997). Although brain retention of liposomes after peripheral administration was observed in some cases, it was due to entrapment within the brain microvasculature (Schackert et al., 1989). Autoradiography in brain revealed no detectable changes in the expression and distribution of β-ARs after intravenous β_1-AS-ODN injection. This provided further evidence that the use of antisense did not have CNS effects.

[0235] High specificity based on gene sequence has made antisense an increasingly useful tool in numerous studies and clinical trials. Its success is manifested by the recent approval of the first antisense drug, Vitavene, by the Food and Drug Administration. However, sequence-independent interactions have also been reported with AS-ODNs. High doses are usually responsible for the nonspecific effects (Chang et al., 1989), but another possible reason is that currently available databases do not cover every gene; thus, homology comparison by BLAST search may no guarantee sequence specificity of AS-ODNs. In this study, β_1-AS-ODN inhibited β_1-AR expression without changing β2-ARs. Although this is likely due to the specificity of the β_1-AS-ODN sequence, other possibilities remain, such as indirect effects on regulatory mechanisms.

[0236] In patients with chronic heart failure, the severity of the disease closely relates to the decrease in cardiac β_1-AR density and functional responsiveness (Brown et al., 1992). In SHRs treated with β_1-AS-ODN, a marked attenuation of the β_1-AR-mediated positive inotropic response in vitro and in vivo, concurrent with the diminished cardiac β_1-AR level. Therefore, these results indicated a positive correlation between cardiac β_1-AR number and functional sensitivity (correlation coefficient >0.90, P<0.01).

[0237] Despite the large decrease in BP, no reflex tachycardia was observed after antisense treatment. However, the suppressive effect of β_1-AS-ODN on Hr was less significant than its negative inotropic response. Several possibilities can be considered. First, β_1-AS-ODN did not affect β_2-ARs, which played an important role in the regulation of HR (Kaumann, 1986; Rodefeld et al., 1996), although it was not involved in the cardiac contraction. Second, antisense inhibition of β_1-AR expression is gradual and less extensive than with β-blockers. It is also possible that β_1-ARs may have a larger reserve for controlling HR than contractility.

5.2 Example 2

Prolonged Reduction in High Blood Pressure with β_1 AR Oligodeoxynucleotides

[0238] Since the introduction of propranolol in 1965, β-blockers have become major first-line drugs for hypertension. Through the inhibition of β-adrenergic receptors in heart and kidney, β-blockers lower high blood pressure via the reduced response to the sympathetic nervous system. However, all current β-blockers have to be taken daily. Also, most have central nervous system side effects that lead to poor patient compliance. Furthermore, the mechanism of β-blockade in hypertension is not well understood (Man in't Veld et al., 1988). Antisense oligonucleotides have been successfully constructed to components of the renin-angiotensin system (RAS) to decrease blood pressure (Phillips et al., 1994). In view of this, novel antisense oligonucleotides targeted to β_1-adrenergic receptors (β_2-ARs), or the brain. Therefore, it is likely to have fewer side effects and longer-lasting action.

[0239] It has been previously shown that antisense oligodeoxynucleotide (β_1-AS-ODN) significantly inhibits β_1-AR expression in the cardiac ventricles, which results in suppressed inotropic response to adrenergic activation and thereby contributes to hypotension. In addition to inducing positive inotropy and chronotropy in the heart, β_1-ARs are also responsible for mediating the sympathetic stimulation of renin expression and secretion from juxtaglomerular cells of the renal cortex. Infusion of isoproterenol has been shown to increase renin expression and secretion and plasma renin activity (PRA) in rats (Holmer et al., 1997). β-Blockers reduce PRA in patients, (Blumenfeld et al., 1999). However, despite the evidence that β-blockers are more effective in patients with high renin profiles (Buhler, 1988), the importance of β-blocker-induced decreases in renin release has been debated (Man in't Veld et al., 1983). The present study investigates whether β_1-AS-ODN reduces renin expression and secretion and whether the RAS is involved in the antihypertensive impact of β_1-AS-ODN could be improved by delivery with cationic liposomes and to determine the optimal charge ratio of liposome:ODN.

[0240] 5.2.1 Methods

[0241] 5.2.1.1 Antisense Sequence and Delivery

[0242] AS-ODN and inverted ODN control were 15-mer and targeted to the AUG start codon of rat β_1-AS-ODN is 5'-CCGCGCCCATGCCGA-3' (SEQ ID NO:197), and the inverted ODN is 5'-AGCCGTACCCGCGCC-3' (SEQ ID NO:198). These ODNs were modified by backbone phosphorothioation. The cationic lipid 1,2-bis(oleoyloxy)-3-(trimethylammonio)propane (DOTAP) mixed with the helper lipid L-α-dioleoyl phosphatidylethanolamine (DOPE, Avanti Polar Lipids) at 1:1 molar ratio was used to deliver ODNs in a single intravenous injection into the tongue vein. ODN-liposome complex was prepared on the day of use by mixing desired amounts of ODNs with DOTAP/DOPE to a final DNA concentration of 300 μg/mL in 5% (wt./vol.) dextrose in water and incubating at room temperature for 60 min.

[0243] 5.2.1.2 Animals

[0244] Adult male SHR (4 to 6 mos. old, Harlan, Indianapolis, Ind.) were kept in cages in a room with a 12 hr. light-dark cycle. Animals were fed standard laboratory rat chow and tap water ad libitum. Tail blood was collected for determination of PRA and angiotensin II (Ang II) levels.

[0245] 5.2.1.3 Blood Pressure Measurement

[0246] Blood pressure was measured by the tail-cuff method as described above. Systolic blood pressure (SBP) was determined as the first pulsatile oscillation on the descending side of the pressure curve. Data values of each rat were taken as an average of ≧4 stable readings. Baseline was determined by averaging 3 days of measurements before antisense administration.

[0247] 5.2.1.4 Membrane Preparation and β-Adrenergic Receptor Binding Assay

[0248] Four days after intravenous injection of saline (n—6) or 0.5 mg/kg inverted ODN (n=6) or 4, 10, 18 and 40 days after injection of 0.5 mg/kg β_1-AS-ODN (n=24), animals were euthanized, and membranes were prepared from the renal cortex of the left kidneys as previously described (Baker and Pitha, 1982). For saturation studies, 100 μg membrane protein was incubated in triplicate with 6 concentrations of [^{125}](−)iodocyanopindolol (ICYP, NEN Life Science, 6.25 to 100 pmol/L) in a total volume of 250 μL containing 50 mmol/L Tris-HCl (pH 7.4) and 5 mmol/L $MgCl_2$ at 36° C. for 60 min. The nonspecific and β_2-adrenergic receptor binding levels were determined in the presence of 1 μmol/L (±)-alprenolol and 150 mmol/L CGP20712A (RBI), respectively. Then the reaction mixture was passed through a Whatman GF/B glass fiber filter with a Brandel harvester, and the bound radioactivity was counted for 1 min.

[0249] 5.2.1.5 Tissue Preparation and Quantitative Autoradiography

[0250] Four days after injection of 0.5 mg/kg β_1-AS-ODN (n=6) or saline (n=6), rats were euthanized, and the right kidneys were removed and frozen in dry ice. Sagittal sections of kidney (20 μm) were cut on a cryostat (Microm) at −20° C. and mounted on microscope slides. Every seventh slide was stained with hematoxylin and eosin for histology. Receptor autoradiography was performed as described (Matthews et al., 1994) with 100 pmol/L ICYP at 25° C. for 150 min. in the presence of 1 μmol/L (−)-propranolol, 100

mmol/L ICI 118,551 (β_2-selective antagonist), or 100 mmol/L CGP 20712A (β_1-selective antagonist) to distinguish nonspecific, β_1, and β_2-bindings. The images were quantified with a computerized image analysis system (MCID, Imaging Research) and normalized with ^{125}I standards. Nonspecific binding was <10% of total binding.

[0251] 5.2.1.6 Reverse Transcription-Polymerase Chain Reaction and Southern Blotting

[0252] At different time points after the single injection of β_1-AS-ODN or inverted ODN, rats were euthanized, and the renal cortex was dissected from the left kidneys, immediately dipped into RNA later tissue storage buffer (Ambion), and stored at −20° C. Total RNA was extracted with RNAwiz reagent (Ambion) and quantified by spectrophotometer. RNA samples from 4 to 5 rats from each time point were pooled. RNA (5 μg) was digested by DNase I and reverse-transcribed by Superscript reverse transcriptase (GIBCO BRL) at 42° C. for 50 min. and 1/20 of the reverse transcription (RT) product was used to run a polymerase chain reaction (PCR) for 20 cycles. PCR primers for β_1-AR were 5'-CTCCGAAGCTCGGCATGG-3' (SEQ ID NO:199) (forward) and 5'-GCACGTCTACCGAAGTCCAGA-3' (SEQ ID NO:200) (reverse) and yielded products of 432 bp, which spanned the AUG start codon. Primers for preprorenin were 5'-AGGCAGTGACCCTCAACATTACCAG-3' (SEQ ID NO:201) (forward) and 5'-CCAGTATGCACAGGTCATCGTTCCT-3' (SEQ ID NO:202) (reverse) and yielded products of 362 bp.

[0253] Primers for GAPDH were 5'-ATCAAATGGGGTGATGCTGGTGCTG-3' (SEQ ID NO:203) (forward) and 5'-CAGGTTTCTCCAGGCGGCATGTCAG-3' (SEQ ID NO:204) (reverse) and yielded products of 505 bp (Jo et al., 1996). RT-PCR products were subjected to Southern blotting, hybridized with psoralen-biotin-labeled cDNA probes, and detected with nonisotopic kits (Ambion). After the membranes had been exposed to x-ray films, the intensity of β_1-AR and preprorenin mRNAs was quantified by densitometry and normalized with GAPDH mRNA levels. The studies were repeated at least twice.

[0254] 5.2.1.7 PRA and Plasma Ang II Levels

[0255] PRA was determined with an angiotensin 1 (1251) radioimmunoassay kit (DuPont). Plasma Ang II levels were measured by radioimmunoassay as previously described (Phillips and Kimura, 1988).

[0256] 5.2.1.8 Statistical Analysis

[0257] Values were expressed as mean±SEM. Differences were considered statistically significant at a value of $P<0.05$. One-way repeated ANOVA and Tukey's test were used to compare blood pressure before and after AS-ODN treatment. Unpaired t test was used to compare B_{max1} PRA, and plasma Ang II levels in 2 groups.

[0258] 5.2.2 Results

[0259] 5.2.2.1 Optimization of β_1-AS-ODN Delivery by Cationic Liposomes

[0260] Systemic delivery of AS-ODN was optimized with the commercially available cationic lipid DOTAP mixed with neutral lipid DOPE. Previous studies reported that a charge ratio of DOTAP:DNA of ≈2.0 achieved the best gene delivery in vivo and in vitro (Yang et al., 1997 and Templeton et al., 1997). Therefore, 5 charge ratios of DOTAP:ODN were tested ranging from 0 to 3.5 to deliver 0.5 mg/kg β_1-AS-ODN intravenously. It was noticed that different batches of liposome mixture varied slightly in structure and particle size, which may influence the delivery efficiency. **FIG. 7** shows the effect of different liposome:ODN charge ratios on blood pressure of SHR (n=6 for each ratio) in a representative experiment. β_1-AS-ODN alone, i.e. at ratio 0, did not change SBP, whereas ratio 0.5 significantly reduced SBP by up to 33 mm Hg for 7 to 8 days. When the ratio was increased, the duration of the hypotensive impact was drastically prolonged to 20 days at ratio 1.5 and 33 days at ratio 2.5 and 3.5, varying with liposome preparations. But the maximum drop in SBP was greater at ratio 1.5 and 2.5 (≈35 mm Hg) than at ratio 3.5 (≈25 mm Hg) (Table 2). Accordingly, the optimal charge ratio of DOTAP:ODN was determined to be 2.5. In the subsequent experiments, SHR (n=24) injected with 0.5 mg/kg β_1-AS-ODN with liposomes at a charge ratio of 2.0 were analyzed for the time course of changes in SBP, receptor levels, and peripheral RAS.

TABLE 4

RANGES OF AMPLITUDE AND DURATION OF REDUCTION IN BLOOD PRESSURE OF SHR AFTER A SINGLE INTRAVENOUS INJECTION OF 0.5 MG/KG β_1-AS-ODN DELIVERED IN DIFFERENT CHARGE RATIOS OF LIPOSOME/ODN

Charge Ratio of Liposome/ODN	Maximum Reduction in Blood Pressure, mm Hg	Range of Reduction, mm Hg	Duration, d (Range
0	2	***	***
0.5	35–38	24–38	7–8
1.5	28–35	18–35	18–20
2.5	30–34	20–34	20–33
3.5	20–24	15–24	20–33

[0261] 5.2.2.2 Effects of β_1-AS-ODN on β-Adrenergic Receptors in Renal Cortex

[0262] Scatchard analysis of β-AR binding in renal cortex (**FIG. 8A**, **FIG. 8B** and **FIG. 8C**) indicated that β_1-AR was the major subtype in the control rats, composing 70% of total β-AR. After β_1-AS-ODN injection, the B_{max} of β_1-ARs was diminished significantly, by 35% on day 4 ($P<0.05$), 29% on day 10 ($P<0.05$), and 23% on day 18, and completely restored on day 40. β_1-AR reduction in kidney coincided with that in heart, and both were accompanied by a significant drop in SBP ($P<0.01$) (**FIG. 8A**). In contrast, the β_2-AR level was not affected (**FIG. 8B**), nor was the affinity of either subtype. Inverted ODN had no effect on either subtype (**FIG. 8C**).

[0263] Kidney slices from the same rats were subject to quantitative autoradiography to display the structural distribution of β-ARs. β_1-Subtype composed ≈60% of the β-AR levels, which was localized predominantly in the renal cortex and the outer band of the medulla. β_2-Subtype was more diffusely distributed in the kidney at a lower level. This result was consistent with previous reports (Summers et al., 1985). Four days after β_1-AS-ODN treatment, the overall density of β_1-subtype in kidney was significantly reduced from 23.5±2.1 to 15.4±3.3 fmol/mg ($P<0.05$). The diminution in renal cortex was particularly conspicuous because of the higher basal level. As expected, the distribution and concentration of β_2-subtype remained unchanged in accord

with binding results. This further confirms the specificity of the inhibitory effect of β_1-AS-ODN on β_1-subtype.

[0264] In an effort to demonstrate whether β_1-AS-ODN decreases the mRNA level of β_1-AR by inducing RNase H digestion, a pair of primers flanking the AUG start codon where β_1-AS-ODN was targeted was used to run a semi-quantatitative RT-PCRTM for 20 cycles, followed by Southern blotting. β_1-AS-ODN did not reduce the level of steady-state β_1-AR mRNA in renal cortex, indicating that the inhibition of β_1-AR expression was not at the transcriptional level.

[0265] 5.2.2.3 Effect of β_1-AS-ODN on Peripheral RAS

[0266] RT-PCR™ revealed that the preprorenin mRNA level in renal cortex was transiently decreased to 62% of control 4 days after β_1-AS-ODN injection. It was completely reversed by day 18 (**FIG. 9A**). Conversely, PRA and plasma Ang II levels showed different patterns of reduction, which were significantly decreased on day 10 and day 18 ($P<0.01$) but not on day 4. Thus, PRA and Ang II seemed to have as delayed action relative to the reduction in renin mRNA (**FIG. 9B**).

[0267] 5.2.3 Summary

[0268] Because β_1-adrenergic receptors are also involved in renin expression and secretion from the kidney, the present study was designed to evaluate the effect of β_1-AS-ODN on peripheral RAS and its contribution to the reduction in blood pressure. In addition, a significant improvement of hypotensive action up to 33 days was achieved by optimizing the delivery of β_1-AS-ODN with cationic liposomes.

[0269] β-blockers have been used to treat hypertension for 3 decades. The reasons for their antihypertensive effects remain largely unclear, but the inhibition of renin release is regarded as a primary mechanism. Many β-blockers can reduce PRA in patients and experimental animals (Blumenfeld et al., 1999 and Holmer et al., 1994). They are found to be more effective in patients with higher renin profiles (Buhler, 1988). In this study, it was found that β_1-AS-ODN effectively decreased PRA and Ang II in the long term. But the decrease in PRA and Ang II did not occur until ≈10 days after β_1-AS-ODN injection, in contrast to the rapid drop in cardiac output 2 days after injection. Thus, it appears that the effects of β_1-AS-ODN on the kidney renin and the circulating RAS are more delayed than cardiac action. Suppression of cardiac output may account for the early phase of the antihypertensive effect of β_1-AS-ODN, whereas the inhibition of renin-angiotensin activity acts as the secondary mechanism underlying the sustained reduction of blood pressure in SHR.

[0270] Receptor binding assay showed that β_1-AS-ODN reduced the β_1-AR levels in renal cortex by ≈30% for 18 days. This is consistent with the decrease of β_1-AR in heart ventricles in magnitude and time course. This suggests that β_1-AS-ODN delivered by cationic liposomes is rapidly transported to peripheral organs after intravenous injection and effectively taken up into heart and kidney cells to a comparable extent. Several mechanisms have been proposed for the AS-ODN inhibition of the expression of target proteins. One involves the decrease in mRNA levels resulting from RNase H digestion of the RNA strand of the RNA-DNA duplex (Phillips et al., 1996 and Phillips et al., 1997). To test this hypothesis, a pair of primers that flanked the AUG start codon of β_1-AR mRNA were designed where AS-ODN bound to perform semiquantitative RT-PCR™. As shown in the Results, there was no reduction in the RT-PCR™ products, indicating the absence of RNase H action. Therefore, the inhibition of β_1-AR expression probably occurs in post-transcriptional steps.

[0271] By decreasing β_1-AR levels in kidney, β_1-AS-ODN significantly reduced PRA and the subsequent plasma Ang II levels. This is unlikely to be through the inhibition of renin expression, however, because there is no long-term diminution of renin mRNA levels. Instead, β_1-AS-ODN may exert its inhibitory impact on renin secretion or the conversion of inactive renin to active renin. This hypothesis is consistent with the observation that β-adrenergic stimulation of renin expression had a time course different from that of renin secretion (Holmer et al., 1997 and Chen et al., 1993). Furthermore, β-blockers have been shown to reduce prorenin processing to active renin without changing total renin (prorenin+PRA) levels in plasma (Blumenfeld et al., 1999).

[0272] Efficient gene delivery is vital to the therapeutic application of AS-ODN in vivo. Among nonviral vectors, cationic liposomes are the most widely used. They are safe, nonimmunogenic, and east to produce on a large scale. However relatively low transfection efficiency has been obtained after intravenous administration, mainly because of the inactivation of cationic liposome by serum. It was recently shown that increasing the charge ratio (±) of liposome to DNA and inducing the maturation of liposome-DNA complex by prolonging incubation time can overcome this problem (Yang et al., 1997; 1998). The optimal charge ratio of DOTAP:DNA was demonstrated to be ≈2 (Yang et al., 1997 and Templeton et al., 1997). Thus, 5 charge ratios were tested ranging from 0 to 3.5 to optimize the AS-ODN delivery. As shown in the results, increasing the charge ratio not only improved the delivery efficiency but also prolonged the duration of β_1-AS-ODN action. The best antihypertensive result (−35 mm Hg for 33 days) was consistently achieved at ratio 2.5.

[0273] In summary, β_1-AS-ODN delivered with cationic liposomes at a single intravenous injection achieves a marked and sustained hypotensive effect (30 to 35 mm Hg for 33 days) in SHR. The β_1-AS-ODN is clearly longer lasting than any current drug, does not inhibit β_2-adrenergic receptors or cross the blood-brain barrier, and has negligible effect on heart rate. Therefore, the antisense is likely to have fewer side effects than currently used P-blockers. Inhibition of cardiac contractility initially followed by reduced renin release is an important mechanism contributing to its antihypertensive effects.

5.3 Example 3

Treatment of Hypertension Using Antisense Compounds

[0274] 5.3.1 Materials and Methods

[0275] 5.3.1.1 Antisense Design and Administration

[0276] AS-ODN and inverted-ODN control were 15 mer and targeted to the AUG start codon of rat β_1-adrenoceptor mRNA (Machida et al., 1990). The sequence of AS-ODN was 5'-CCGCGCCCATGCCGA-3' (SEQ ID NO: 1), and the

sequence of the inverted-ODN was 5'-AGCCGTAC-CCGCGCC-3' (SEQ ID NO:138). This AS-ODN was chosen from six AS candidates targeted to different regions of β_1-adrenoceptor mRNA, based on the intensity of cardiac β_1-AR inhibition and reduction of blood pressure in SHR. These oligonucleotides were modified by backbone phosphorothioation. ODNs delivered with cationic liposomes were injected into tongue vein.

[0277] 5.3.1.2 Preparation of Liposomes and ODN/Liposome Complex

[0278] The cationic lipid 1,2-bis(oleoyloxy)-3-(trimethylammonio)propane (DOTAP) was mixed with a helper lipid L-α dioleoyl phosphatidylethanolamine (DOPE, Avanti Polar Lipids) at 1:1 mole ratio, briefly sonicated and stored at 4° C. until use. The average diameter of liposome is 200-350 nm (Tang and Hughes, 1998). ODN/liposome complex was prepared on the day of use by mixing desired amounts of ODNs with DOTAP/DOPE to the final DNA concentration of 300 μg/ml in 5% (wt./vol.) dextrose in water and incubating at room temperature for 60 min. Two DNA/lipid mole ratios, i.e., 1:0.5 and 1:2.5, were used in each study.

[0279] 5.3.1.3 Animal Surgery

[0280] Adult male SHRs (250-350 g, Harlan) were kept in cages in a room with a 12-hr light-dark cycle. Animals were fed standard laboratory rat chow and tap water ad libitum.

[0281] 5.3.1.4 Telemetric Sensor Implantation

[0282] Before implantation, the zero of each radiotransmitter (TA11PA-C40, Data Sciences) was verified to be ≦4 mm Hg. SHR were anesthetized with 100 mg/kg ketamine and 15 mg/kg xylazine and a midline abdominal incision was made. A fluid-filled catheter was then inserted into the right femoral artery and the tip of the catheter was in the abdominal aorta caudal to the renal arteries. The implanted rats were allowed to recover for one week.

[0283] 5.3.1.5 Jugular Vein Cannulation

[0284] One week after telemetric implantation, rats were anesthetized and a curved catheter made of PE 50 and vinyl tubing was inserted into the external branch of the jugular vein. The tubing was led under the skin of the neck and exposed on the back to allow for drug infusion. Rats were allowed to recover for 24 hr before experimentation. The catheters were flushed with 100 U heparin every day to prevent clogging.

[0285] 5.3.1.6 Membrane Preparation and β-AR Binding Assay

[0286] Four days after intravenous injection of saline (n—6) or 1 mg/kg inverted ODN (n=6), or 2, 4, 10, 18 days after injection of 1 mg/kg β_1-AS-ODN (n=24), animals were sacrificed and membranes were prepared from heart ventricles as previously described (Baker and Pitha, 1982). For saturation studies, 100 μg membrane protein was incubated in triplicate with six concentrations of ^{125}I-(−)iodocyanopindolol (1-CYP, NEN Life Science, 6.25-100 pM) in a total volume of 250 μl containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ at 36° C. for 60 min. The nonspecific and β_2-adrenoceptor binding levels were determined in the presence of 1 μM (±)-alprenolol and 150 nM CGP20712A (RBI), respectively. Then the reaction mixture was passed through Whatman GF/B glass fiber filter using Brandel harvester and the bound radioactivity was counted for one min.

[0287] 5.3.1.7 Tissue Preparation and Quantitative Autoradiography

[0288] Four days after injection of 1 mg/kg β_1-AS-ODN (n=6) or saline (n=6), rats were sacrificed and tissues were removed and frozen in dry ice. Coronal sections of brain, horizontal sections of heart and sagittal sections of kidney (20 μm) were cut on a cryostat (Microm) at −20° C. and mounted on microscope slides. Every seventh slide was stained with haematoxylin and cosin for histology. Tissue sections were preincubated in Krebs buffer (NaCl 118.4 mM, KCl 4.7 mM, $MgSO_4$ 1.2 mM, $CaCl_2$ 1.27 mM, NaH_2PO_4 10.0 mM, pH 7.1) containing 0.1 mM guanosine triphosphate (GTP), 0.1 mM ascorbic acid and 10 μM phenylmethylsulfonylfluoride (PMSF) for 30 min at 25° C. Sections were then incubated in Krebs buffer containing 0.1 mM ascorbic acid and 10 μM PMSF with 100 pM I-CYP at 25° C. for 150 min, in the presence of 1 μM (−)propranolol, 100 nM ICI118,551 (β_2-selective antagonist) or 100 nM CGP20712A (β_1-selective antagonist) to distinguish nonspecific, β_1- and β_2-bindings. Labeled sections were rinsed in the same buffer, followed by two 15-min washes at 37° C. in the buffer and rinsed in distilled water at 25° C. (Matthews et al., 1994). Dried sections were then exposed to X-ray films (Kodak Biomax-MR). The images were quantitated with a computerized image analysis system (MCID, Imaging Research) and normalized using ^{125}I-standards. Nonspecific binding was less than 10% of total binding.

[0289] 5.3.1.8 Determination of Effects of β_1-AS and Atenolol on Cardiovascular Parameters in Response to β-Stimulation

[0290] 5.3.1.8.1 Langendorff Heart Perfusion

[0291] Forty-eight hr after injection of 1 mg/kg β_1-AS-ODN (n=9) or inverted-ODN (n=6), SHR were anesthetized and sacrificed. Hearts were quickly removed and perfused via the aorta with oxygenated Krebs buffer (118 mM NaCl, 18.75 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.25 mM $CaCl_2$, 1.1 mM Glucose, 0.01 mM EDTA) at a constant flow of 7.0 ml/min at 36° C. Coronary perfusion pressure (CPP) was measured via a catheter placed proximal to the aorta and connected to a pressure transducer (Gould Statham P231D). A latex balloon filled with water and connected to the pressure transducer was inserted into the left ventricle through the left atrium to measure left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), and developed left ventricular pressure (dLVP) (dLVP=LVSP−LVEDP). LVEDP during equilibration was set at 5 to 7 mmHg. CPP, LVEDP and LVSP were continuously recorded on a 4-channel recorder (Astro-Med). After baselines for dLVP and heart rate, (HR) were stable for 5 min, isoproterenol (ISO, non-specific β-agonist) was given at 0.01, 0.025, 0.05, 0.12 μM at 10-min intervals so as to avoid the effect of tachyphylaxis.

[0292] 5.3.1.8.2 Telemetric Monitoring of Live Animals

[0293] The effects of β_1-AS-ODN and atenolol on cardiac dP/dt_{max}, HR and systolic blood pressure (SBP) were compared in the same group of SHR (n=4). Two days after catheterization of jugular vein, control values were taken

and 1 mg/kg β_1-AS-ODN was injected. Forty-eight hr later, rats were tested for the effect of β_1-AS-ODN. The rats were allowed to recover until all the cardiovascular parameters returned to control values. Then 1 mg/kg atenolol (β_1-selective antagonist) was injected and rats were tested 30 min later. For β_1-stimulation, SHR were infused with dobutamine (β_1-selective agonist) through jugular vein catheter at 5, 10, 20, 40 μg/kg/min. Each dose was given for 5 min continuously and at 1-hr intervals until all cardiovascular parameters returned to baseline so as to avoid the effect of tachyphylaxis. BP and HR were sampled every min. dP/dt_{max} was calculated from the slope of the rising pulse pressure curve and determined every min. The difference between values at each dose and baseline was denoted as Δ.

[0301] 5.3.2 Results

[0302] 5.3.2.1 Effect on Cardiac β-Adrenoceptor Density

[0303] A single intravenous injection of 1 mg/kg β_1-AS-ODN delivered with cationic liposomes at a 1:2.5 mole ratio significantly reduced the β_1-adrenoceptor densities in the SHR hearts for 18 days ($P<0.01$). The drop was at its maximum of 47% on day 4, 33% on day 10, and maintained at 29% on day 18. In contrast, there was no significant change in the B_{max} of β_2-adrenoceptors. K_D of both subtypes remained unaltered (Table 5). Consequently, the β_1/β_2 subtype ratio in the ventricles was diminished from ~70/30 to ~50/50 by β_1-AS-ODN. Inverted-ODN had no effect on either subtype.

TABLE 5

B_{MAX} AND K_D OF β_1-AND β_2-ADRENOCEPTORS IN CARDIAC VENTRICLES OF SHR 4 DAYS AFTER TREATMENT WITH SALINE, INVERTED-ODN OR β_1-AS-ODN

	Total (β_1 + β_2)		β_1		β_2	
	K_D (pM)	B_{max} (fmol/mg)	K_D (pM)	B_{max} (fmol/mg)	K_D (pM)	B_{max} (fmol/mg)
Saline	55.4 ± 6.7	27.6 ± 0.9	64.7 ± 5.4	18.7 ± 0.9	29.6 ± 0.5	9.1 ± 0.6
Inverted-ODN	57.1 ± 3.5	26.3 ± 3.0	66.0 ± 1.0	18.1 ± 2.3	32.0 ± 2.4	8.7 ± 0.5
β_1-AS-ODN	37.0 ± 1.0*	19.8 ± 1.8*	60.5 ± 3.4*	10.0 ± 1.2*	30.7 ± 3.3	9.9 ± 0.5

Data represent mean ± SEM of each group (N = 6–10).
*P < 0.01 versus saline control.

[0294] 5.3.1.9 Blood Pressure Monitoring

[0295] 5.3.1.9.1 Telemetry

[0296] Each rat cage was placed on a receiver (RLA1020, Data Sciences) for measurement of cardiovascular parameters. Data were collected with a computer-based data acquisition program (Dataquest LabPRO3.0; Data Sciences). BP and HR were measured every 10 min and averaged every 1 to 24 hr. Before treatment, SHR were monitored for a week to get a stable baseline.

[0297] 5.3.1.9.2 Tailcuff

[0298] Rats were warmed for 20-30 min in cages on heating pads. The temperature was controlled at 35-37° C. Then rats were placed in a plastic restrainer kept at 37° C. A pneumatic pulse sensor was attached to the tail. After cuff inflation, SBP was determined as the first pulsatile oscillation on the descending side of pressure curve. HR was determined by manual counting of pulse numbers per unit time. BP and HR were recorded by a Narco physiograph. Data values of each rat were taken as an average of at least four stable readings. Baseline was determined by averaging three days of measurements before antisense administration.

[0299] 5.3.1.10 Statistic Analysis

[0300] Values were expressed as mean±SEM. Difference was considered statistically significant at $P<0.05$. Unpaired t-test was used to compare B_{max}, dLVP and BP in two groups. One way repeated ANOVA and Tukey test were used to compare $\Delta dP/dt$ and AHR upon dobutamine infusion in different groups. Pearson product moment correlation was used to assess the relationship between β_1-adrenoceptor B_{max} and dLVP.

[0304] 5.3.2.2 Effect on Cardiac Contractility and Heart Rate in Response to β-Stimulation

[0305] Forty-eight hr after injection of 1 mg/kg β_1-AS-ODN, the cardiac inotropic and chronotropic responses to β-stimulation were determined in SHR in vitro and in vivo.

[0306] First, isolated hearts were perfused with incrementing doses of ISO, which enhanced heart rate and contractility via activating β-adrenoceptors. The dLVP-ISO dose-response curve, which reflected the positive inotropic effect of ISO, was significantly shifted down by β_1-AS-ODN ($P<0.02$). Heart rate was not significantly decreased.

[0307] In vivo test was performed in conscious SHR monitored by radiotelemetry. β_1-AS-ODN significantly ($P<0.02$) dampened the increase in dP/dt_{max} in the face of dobutamine (β_1-selective agonist). The change in heart rate was not significantly reduced, which echoed the results in isolated, perfused hearts. On the other hand, the response of blood pressure to dobutamine was biphasic. Dobutamine elevated SBP by 5-8 mmHg at low infusion speed and reduced SPB at higher speed. This is probably due to the partial β_2-argonistic activity of dobutamine at high doses and the consequent vasodilatory effect on blood pressure. Relative to β_1-AS-ODN, 1 mg/kg atenolol produced a more profound decline in $\Delta dP/dt_{max}$ and AHR during a 5-hr period of time after injection. Moreover, it reduced the resting dP/dt_{max} (2450±295 mmHg/sec versus control 2937±277 mmHg/sec) and caused bradycardia (295±12 bpm versus control 365±8 bpm) ($P<0.05$), while β_1-AS did not change resting contractility (2922±249 mmHg/sec) or heart rate (365±12 bpm). But the effects of atenolol on $\Delta dP/dt_{max}$ and

ΔHR were transient. Within 24 hr after atenolol administration, the inotropic and chronotropic effects of dobutamine had returned to control level.

[0308] 5.3.2.3 Effect on Blood Pressure of SHR

[0309] The effects of single injection of 1 mg/kg β_1-AS-ODN on blood pressure of SHR measured by tailcuff method were observed. AS-ODN was delivered with cationic liposome at different mole ratios of DNA/lipid, ie., 1:0.5 and 1:2.5. β_1-AS-ODN delivered with liposomes at 1:0.5 ratio diminished SBP for 8 days. The maximum drop was 38±5 mmHg. When the mole ratio was increased to 1:2.5, this hypotensive effect was drastically prolonged to 20 days. No effect was seen with inverted-ODN.

[0310] In order to compare the effects of β_1-AS-ODN and atenolol, radiotelemetry was used to monitor blood pressure and heart rate on a regular basis. One mg/kg β_1-AS-ODN delivered with liposomes at a 1:0.5 ratio produced a maximum drop of 15 mmHg in mean blood pressure. The antihypertensive effect lasted for 8 days, which was consistent with the results measured with tailcuff. Heart rate was not significantly altered. In contrast to AS-ODN, although the onset of hypotensive effect caused by 1 mg/kg atenolol occurred as early as 20 min after injection, it lasted for only 10 hr. In addition, atenolol caused considerable bradycardia up to an average of −75 bpm.

[0311] 5.3.2.4 Effect on β-Adrenoceptor Distribution in Brain, Heart and Kidney

[0312] Quantitative autoradiography of 6-18 tissue slices in brain, heart and kidney was analyzed four days after intravenous β_1-AS-ODN administration. No changes in the distribution of β-adrenoceptors in forebrain and brainstem regions were detected. This indicated the absence of antisense effect on the β-adrenoceptor expression in CNS. However, β_1-AS-ODN significantly ($P<0.05$) reduced β_1-adrenoceptor densities in cardiac ventricles (from 30.2±2.1 to 20.6±2.5 fmol/mg) and renal cortex (26.4±3.1 to 17.4±3.3 fmol/mg). This was consistent with the binding results. β_2-adrenoceptors were not affected in any tissues.

[0313] 5.3.3 Summary

[0314] This example demonstrates the effects of β_1-AS-ODN compositions on high blood pressure in a model of hypertension as compared to using a traditional β-blocker modality. β_1-AS-ODNs knocked down β_1-adrenergic activity, resulting in long-term attenuation of blood pressure. The results indicated that β_1-AS-ODN reduced β_1-adrenoceptor density and cardiac contractility following β_1-stimulation in vitro and in vivo and lowered high blood pressure of SHR. These findings are consistent with the hypothesis that β_1-AS, through the inhibition of β_1-adrenoceptor expression, is able to render heart, kidney and other tissues less sensitive to sympathetic activation, which is a major contributing factor in high blood pressure. A single injection of β_1-AS-ODN effectively decreased the cardiac β_1-adrenoceptor density, which was accompanied by diminished ventricular contractility and cardiac output in response to β-stimulation. The antihypertensive effect in SHR was up to a 38-mmHg reduction lasting as long as 20 days.

[0315] Treatment of β_1-AS-ODN reduced cardiac β_1-adrenoceptor density by ~50% in four days. There is considerable variation reported in the literature on the half-life of β-adrenoceptors (Baker and Pitha, 1982; Neve and Molinoff, 1986; Winter et al., 1988). From the results with the AS-ODN inhibition, it appears that the half-life of cardiac β_1-adrenoceptor is approximately 2-4 days to allow for 50% reduction of β-adrenoceptor density within 4 days.

[0316] Both tailcuff and telemetry measures of blood pressure showed a significant drop after antisense treatment. SHR responded to β_1-AS-ODN to a greater degree of hypotension when subjected to tailcuff versus telemetry. In addition, the baseline measured with tailcuff was consistently higher than that with telemetry by 20-30 mmHg in the same rats. Bazil et al. (1993) reported a similar phenomenon. They compared the cardiovascular parameters recorded by telemetry, tailcuff and arterial catheter and observed a more sensitive hypotensive effect of captopril with tailcuff. Tailcuff measurement of blood pressure involves warming and restraint of rats. It is conceivable that β_1-AS, through the suppression of sympathetic activity, can decrease blood pressure of animals under stress more effectively.

[0317] Cationic liposomes are effective vehicles for gene delivery. It has been shown that liposome entrapment not only improves the cellular uptake of DNA, but also protects DNA from degradation and extends its circulation time (Allen, 1997; Liu et al., 1997). Numerous factors influence the efficiency of cationic liposome-mediated intravenous gene delivery, such as DNA/lipid ratio, selection of lipids and preparation procedure (Liu et al., 1997; Yang and Huang, 1998). A widely used lipid formula DOTAP/DOPE was used to deliver β_1-AS-ODN at four mole ratios (1:0.5, 1:1.5, 1:2.5, and 1:3.5). The optimal ratio 1:2.5 was determined based on the duration and magnitude of the antihypertensive effects. A profound and prolonged fall in blood pressure of 38 mmHg up to 20 days was achieved at this ratio, which followed the reduction in β_1-adrenoceptor binding to a maximum of 47% at day 4, 33% at day 10, and 29% at day 18. This implies that β_1-AS-ODN effectively inhibits the functionally active receptors involved in the blood pressure.

[0318] The blood-brain barrier formed by capillary endothelia is only permeable to small lipophilic molecules with molecular weight <600 Da (Padridge, 1998). Owing to their high hydrophilicity, ODNs undergo negligible transport through blood-brain barrier and have very limited access to CNS (Agrawal et al., 1991). The cellular and organ distributions of DNA/liposome complexes with fluorescent labeling were previously studied in mice after intravenous injection and the results indicated that the complexes were primarily taken up by capillary endothelial cells in most of the peripheral organs including lung, heart, kidney and spleen, but absent in the brain (McLean et al., 1997). Although the brain retention of liposomes after peripheral administration was observed in some cases, it is due to entrapment within the brain microvasculature (Schackert et al., 1989). In this study, autoradiography in brain revealed no detectable changes in the expression and distribution of β-adrenoceptors after intravenous β_1-AS-ODN injection. This provided further evidence that an antisense modality does not produce CNS effects.

[0319] High specificity based on gene sequence has made antisense an increasingly useful tool in numerous studies and clinical trials. Its success is manifested by the recent approval of the first antisense drug Vitrovene by FDA. In the

present study, β_1-AS-ODN inhibited β_1-AR expression without changing β_2-adrenoceptors, demonstrating a specificity of the β_1-AS-ODN selected sequences.

[0320] In patients with chronic heart failure, the severity of the disease closely relates to the decrease in cardiac β-AR density and functional responsiveness (Brown et al., 1992). In SHR treated with β_1-AS-ODN, a marked attenuation of the β_1-adrenoceptor-mediated positive inotropic response was observed in vitro and in vivo, concurrent with the diminished cardiac β_1-AR level. Therefore, these results indicated a positive correlation between cardiac β_1-AR number and functional sensitivity (correlation coefficiency >0.90, P<0.01).

[0321] Despite the large decrease in blood pressure, no reflex tachycardia was observed after antisense treatment. However, the suppressive effect of β_1-AS-ODN on heart rate was less significant than its negative inotropic response. The reason for this is still under investigation. Several possibilities can be considered. First, β_1-AS-ODN did not affect β_2-adrenoceptors, which played an important role in the regulation of heart rate (Kaumann, 1986; Rodefeld et al., 1996), while not involved in the cardiac contraction. Second, antisense inhibition of β_1-AR expression is gradual and to a lesser extent than β-blockers. It is also possible that β_1-ARs may have a larger reserve for controlling heart rate than contractility. Finally, evidence suggests that cardiomyocytes preferentially take up AS-ODN while pacemaker cells are less efficient.

[0322] The cardiovascular effects of β_1-AS-ODN were compared with a hydrophilic β_1-selective antagonist, atenolol. β_1-AS-ODN showed advantages over atenolol in reducing blood pressure and maintaining normal heart rate. Although the onset of β_1-AS-ODN action was slower than atenolol, it lasted much longer, 20 days compared to less than 1 day with atenolol. Furthermore, β_1-AS-ODN did not affect heart rate, while atenolol caused appreciable bradycardia. Bradycardia is a common complaint by patients taking this drug. Atenolol also reduced resting ventricular contractility and heart rate and thereby reduced resting cardiac output. β_1-AS-ODN is unlikely to alter resting cardiac performance. The results presented here suggest that β_1-AS-ODN offer a significant improvement over currently used β-blockers, in both prolonged blood pressure reduction and absence of effects on β_2-adrenoceptors and CNS.

5.4 Example 4

Protection Against Myocardial Ischemia-Reperfusion-Induced Cardiac Dysfunction by Antisense Compositions Directed at β_1-AR mRNA

[0323] Acute myocardial ischemia causes significant increase in plasma catecholamine levels, which leads to exacerbation of the ischemic myocardial injury (Waldenstrom et al, 1978; Rona, 1985). The worsening myocardial ischemia is an important factor in cardiac dysfunction. Acute myocardial ischemia is also characterized by increased sensitivity of β_1-ARs in the myocardium during acute ischemia (Strassere et al, 1990). β-ARs form the interface between the sympathetic nervous system and the cardiovascular system (Strassere et al, 1990; Mukherjee et al., 1979; Maisel et al., 1985. Importantly, β_1-AR subtype 1 (β_1-AR) is by far the predominant β-AR in myocardium (Minneman et al., 1995) and its activity and sensitivity are believed to regulate cardiac function via adenylyl cyclase activity (Mukherjee et al., 1979; Thandroyen et al., 1986; Bohm, 1995). Several studies have shown that density of β_1-AR increases and the expression of β_1-AR mRNA is augmented in the myocardium after acute ischemia (Maisel et al., 1985; Karliner et al., 1989; Ihl-Vahl et al., 1995). Experimental and clinical results have also demonstrated that β_1-AR blockade, especially selective blockade of β_1-AR, can protect myocardium against ischemic injury and cardiac dysfunction (Schulz et al., 1995; Ablad et al., 1987; Lu et al., 1990), decrease infarct size (Schulz et al., 1995), and reduce the incidence of sudden cardiac death in patients with myocardial infarction (Yusuf et al., 1985).

[0324] Although chemical β-AR blockers are commonly used in the treatment of ischemic heart disease, these agents often cause central nervous system side effects and β_2-AR antagonistic activity is associated with increase in peripheral vascular resistance. Development of antisense-oligodeoxynucleotides (AS-ODNs) against specific receptor mRNA is a novel approach to decrease the synthesis of receptor proteins (Phillips et al., 1996; Dachs et al., 1997). This approach has potential to be of therapeutic benefit in disease states characterized by upregulation of these receptors (Phillips et al., 1996; Dachs et al., 1997; Yang et al., 1998). A recent study indicated that AS-ODNs directed at angiotensin II type 1 receptors (AT_1) decrease the synthesis of AT_1 receptor protein and protects the ischemic rat heart from the adverse effects of ischemia (Yang et al., 1998). As demonstrated herein, a single intravenous injection of an antisense compositions directed against β_1-AR mRNA can reduce blood pressure in SHR for periods of at least 20 days (Zhang et al., 1999).

[0325] In this example, the effect of AS-ODNs on lipid peroxidation was examined. Also examined were their effects on the expression of β_1-AR protein and mRNA in the myocardium after ischemia-reperfusion.

[0326] 5.4.1 Materials and Methods

[0327] 5.4.1.1 Antisense Compositions and Liposomal Formulations

[0328] AS-ODNs and inverted-oligodeoxynucleotides (IN-ODNs) control were 15-mers and targeted to −5 to +10 of rat β_1-AR mRNA encompassing the AUG start colon. The sequence of AS-ODNs was 5'-CCGCGCCCATGCCGA-3' (SEQ ID NO: 1), and the corresponding IN-ODNs was 5'-AGCCGTACCCGCGCC-3' (SEQ ID NO:138) (Zhang et al., 1999). These ODNs were modified by backbone phosphorothioation and synthesized in the DNA Synthesis Core Laboratory of the University of Florida.

[0329] Since cationic liposomes enhance the uptake of DNA by cells and also protect DNA from degradation and extend its circulation time, DOTAP/DOPE (mole:mole=1:1) liposomes were used to deliver the antisense compositions to host animals. The average diameter of liposomes was 200-300 nm. ODNs/liposomes complex was prepared on the day of use by mixing desired amount of ODNs with DOTAP/DOPE to final DNA concentration of 300 μg/ml in 5% (wt./wt.) dextrose in water and incubating at room temperature for 60 min (Yang et al., 1998; Zhang et al., 1999).

[0330] 5.4.1.2 Animals

[0331] Male Sprague-Dawley rats weighing 200-250 g were injected intravenously with either AS-ODNs (n=7) or IN-ODNs (n=7) at a dose of 200 μg/rat 4 days before excising the hearts. DOTAP/DOPE liposomes (700 μg/rat) were given along with ODNs. Parallel groups of rats were treated with saline (n—13), or the selective β_1-AR blocker atenolol 2 mg/kg (n=7) 6 hr before the hearts were excised.

[0332] 5.4.1.3 Isolated Perfused Heart Model

[0333] Four days after administration of AS-ODNs or IN-ODNs or 6 hr after administration of atenolol or saline, rats were anesthetized with sodium pentobarbital (40 mg/kg) intraperioneally. The hearts were excised rapidly and placed in ice-cold Kreb-Henseleit buffer (mmol/L: NaCl 118, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.25, $NaHCO_3$ 25, and glucose 11, pH 7.4). Within one min, the hearts were transferred to an isolated perfusion apparatus and perfused via the aorta with oxygen-saturated (95% O_2+5% CO_2) Kreb-Henseleit buffer kept at 37° C. with the use of a MasterFlex pump (model 7015-21, Cole-Palmer Instrument Co.) according to the modified Langendroff procedure (Yang et al., 1998; Neely and Rovetto, 1975). The heart was placed in a semi-closed circulating water-warmed (37° C.) air chamber, paced atrially with a Medtronic 5320 pacemaker at a rate of 300 bpm, and perfused at a constant flow (5.5-6.0 ml/per min). Coronary perfusion pressure (CPP) was measured via a catheter placed just proximal to the aorta and connected to a Gould Statham P23ID pressure transducer. A latex balloon filled with water and connected to a Gould Statham P23ID pressure transducer was inserted in the left ventricle through the left atrium to measure left ventricular end-diastolic pressure (LVEDP), left ventricular systolic pressure (LVSP), and developed left ventricular pressure (dLVP) (dLVP=LVSP−LVEDP). LVEDP during equilibration was set at 5 to 7 mmHg. All measurements were continuously recorded on a 4-channel record (Astro-Med).

[0334] 5.4.1.4 Myocardial Ischemia and Reperfusion

[0335] Six hearts from saline-treated rats were continuously perfused with Kreb-Henseleit buffer for 80 min and served as sham control. Hearts from other rats, after 20 min of equilibration, were subjected to 30 min of ischemia followed by 30 min of reperfusion. After completion of the study, hearts were frozen in liquid-nitrogen for β_1-AR analysis by binding assay, β_1-AR protein analyses by Western blot, β_1-AR mRNA analysis by reverse transcription-polymerase chain reaction (RT-PCR™), and measurement of MDA.

[0336] 5.4.1.5 Determination of β_1-AR Density in Myocardium

[0337] Membrane protein was prepared from left ventricles as previously described (Baker and Pitha, 1982). For saturation studies, 100 μg membrane protein was incubated in triplicate with ^{125}I-(−)iodocyanopindolol (1-CYP, NEN Life Science, 6.25-100 pM) in a total volume of 250 μl containing 50 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$ at 36° C. for 60 min. The nonspecific and P_2-adrenoceptor binding were determined in the presence of 1 μM (±)-alprenolol and 150 mM CGP207ASA (RBI), respectively. Then the reaction mixture was passed through Whatman GF/B glass filter using Brandel harvester and the radioactivity was counted for one min.

[0338] 5.4.1.6 Quantification of β_1-AR Protein Expression in Myocardium

[0339] Myocardial tissues were homogenized and lysed in boiling lysis buffer (1% SDS, 0.1% Triton X-100®, and 10 mmol/L Tris-HCl, pH 7.4) and centrifuged at 10,000 RPM for 30 minutes at 4° C. The lysate protein from myocardial tissues (20 μg/lane) was separated by 8% SDS-PAGE using a Bio-Rad Mini-Protean cell, transferred to nitrocellulose membrane (Amersham). After incubation in blocking solution (4% non-fat milk, Sigma), membranes were incubated with 1:1000 dilation primary antibody (polycolonal antibody to β_1-AR, Santa Cruz Biotechnology) for overnight at 4° C. Membranes were washed and incubated with 1:2000 dilution second antibody (Amersham) for one hr. The membranes were detected with the ECL system, as described previously (Yang et al., 1998; Li et al., 1999).

[0340] 5.4.1.7 Determination of β_1-AR mRNA

[0341] Total RNA was isolated from rat myocardium with the single step acid-guanidinum thiocyanate-phenol-chloroform method and quantified (Chomczynski and Sacchi, 1987). One μg of total RNA was reverse transcribed with oligo-dT (Promega) and M-MLV reverse transcriptase (Promega) at 37° C. for one hr. 1.5 μl of RT material was amplified with Taq DNA polymerase (Promega) using a primer pair specific to β_1-AR:

[0342] Forward primer: 5'-CTCCGAAGCTCGGCATGG-3' (SEQ ID NO: 139); and

[0343] Reverse primer: 5'-GCACGTCTACCGAAGTCCAGA-3' (SEQ ID NO: 140).

[0344] PCR™ product was 432 base pairs. For PCR, 35 cycles were used at 95° C. for one minute, 60° C. for one minute, and 72° C. for one min. The RT-PCRTM amplified samples were visualized on 1.8% agarose gels using ethidium bromide. A primer pair of rat GAPDH was used as control:

[0345] Forward primer: 5'-ATCAAATGGGGTGCTGGTGCTG-3' (SEQ ID NO:141); and

[0346] Reverse primer: 5'-CAGGTTTCTCCAGGCGGCATGTCA-3' (SEQ ID NO:142).

[0347] For PCR™, 35 cycles were used at 95° C. for one min, 60° C. for one min, 72° C. for one min. PCR™ product was 504 base pairs. Relative intensity of bands of interest were analyzed by NSF-300G Scanner (Microtek) (Yang et al., 1998; Li et al., 1999).

[0348] 5.4.1.8 Determination of MDA Levels in Myocardium

[0349] Malondialdehyde (MDA) levels in myocardium were measured in duplicate by a modification of the method of Ohkawa et al., (1979). Briefly, the ventricular tissues were homogenized. The assay mixture consisted of 0.1 ml of the tissue homogenate, 0.4 ml of 0.9% NaCl, 0.5 ml of 3% sodium dodecylsulfate, 3 ml of TBA (thiobarbituric acid reagent, containing equal parts of 0.8% aqueous thiobarbituric acid and acetic acid) and was heated for 75 min at 95° C. Thereafter, 1 ml cold 0.9% NaCl was added to the mixture, which was cooled and extracted with 5 ml n-butanol. After centrifugation at 3,000 rpm for 15 min, the butanol phase was assayed spectrophotometrically at 532 nm. Tetramethoxypropane (in amounts of 0, 0.1, 0.2, 0.4, 0.8, 1.0 mmole) served as external standard. MDA levels in myocardium were expressed in μmol/g tissue.

[0350] 5.4.1.9 Data Analysis

[0351] Data was presented as mean±SD. Statistical significance was determined in multiple comparisons among independent groups of data in which ANOVA and the Student-Newman-Keuls test indicated the presence of significant differences. A P value of $\leqq 0.05$ was considered statistically significant.

[0352] 5.4.2 Results

[0353] 5.4.2.1 Cardiac Dysfunction During Ischemia-Reperfusion

[0354] The basal values of CPP, LVSP, LVEDP, and dLVP were similar in all groups of rat hearts. In the control continuously buffer-perfused hearts observed for 80 min, there were only minimal (~5%) changes in the indexes of cardiac function. In the hearts from saline-treated rats, 30 min of ischemia followed by 30 min of reperfusion resulted in marked cardiac dysfunction, indicated by a significant increase in CPP and LVEDP, and a decrease in LVSP and dLVP (all $P<0.01$, vs. pre-ischemia values).

[0355] Treatment of rats with AS-ODNs markedly attenuated the ischemia-reperfusion-induced myocardial dysfunction, indicated by preservation of LVSP and dLVP and minimization of increase in LVEDP and CPP (all $P<0.01$, vs. saline group). Treatment of rats with atenolol also reduced the increase in CPP and LVEDP induced by ischemia-reperfusion (all $P<0.05$, vs. saline group, n=7), and modestly attenuated the ischemia-reperfusion-induced change in LVSP and dLVP ($P<0.05$ vs. saline group, n=7). AS-ODN treatment was more effective than atenolol treatment in preserving dLVP after ischemia-reperfusion ($P<0.05$). Treatment with IN-ODNs showed no effect on ischemia-reperfusion-induced myocardial dysfunction.

[0356] 5.4.2.2 MDA Levels in Myocardium

[0357] MDA levels in myocardium increased significantly after ischemia-reperfusion ($P<0.05$ vs. sham control hearts, n=6). Pretreatment of rats with AS-ODNs and atenolol attenuated the increase in MDA levels in the myocardium (both $P<0.05$ versus saline pretreatment, n=7 each group). As expected, IN-ODNs did not affect MDA levels in myocardium.

[0358] 5.4.2.3 Change of β_1-AR Density in Myocardium After Ischemia-Reperfusion

[0359] Myocardium from sham control continuously perfused hearts continuously exhibited β_1-AR and β_2-AR (β_1-AR>>β_2-AR). After ischemia-reperfusion in the saline-treated rat hearts, there was a consistent increase in β_1-AR density in myocardium (B_{max} 28.7±5.4 vs. 19.6±1.7 fmol/mg in continuously perfused buffer-perfused rat hearts, $P<0.05$, n=4), while there was no change in β_2-AR density. Treatment of rats with AS-ODNs resulted in a decrease in β_1-AR density in the ischemic-reperfused myocardium (B_{max} 15.9±1.3 vs. 28.7±5.4 fmol/mg in saline-treated rat hearts, $P<0.05$, n=4) while there was no change in β_2-AR density. Treatment of rats with INV-ODNs or atenolol had no effect on β_1-AR density in the ischemic-reperfused myocardium.

[0360] 5.4.2.4 Express of β_1-AR Protein and mRNA in Myocardium

[0361] Western analysis of the control continuously perfused hearts showed a distinct β_1-AR protein band of 41 kDa. Similar molecular weight band was observed in hearts from saline-, AS-ODNs-, IN-ODNs- and atenolol-treated rat hearts. The β_1-AR protein band was very dense in the saline-treated rat hearts, including upregulation of the protein during ischemia-reperfusion. Treatment of rats with AS-ODNs abolished the ischemia-reperfusion-mediated increase in β_1-AR protein expression. Notably, treatment of rats with IN-ODNs or atenolol had no effect on the density of β_1-AR protein.

[0362] Ischemia-reperfusion also resulted in an increase of mRNA for β_1-AR signal (adjusted for GAPDH signal) by myocardium of saline-treated rats, as determined by RT-PCR. Pretreatment of rats with AS-ODNs attenuated the increase of mRNA for β_1-AR in myocardium, but mRNA level for β_1-AR in the myocardium was not significantly affected by treatment of rats with IN-ODNs or atenolol.

[0363] 5.4.3 Summary

[0364] The present example demonstrates the protective role of AS-ODNs directed at β_1-AR mRNA against cardiac dysfunction after a brief period of ischemia-reperfusion. The study compared the effects of pretreatment of rats with AS-ODNs directed at β_1-AR mRNA or a selective β_1-AR blocker atenolol in this process. This study showed that ischemia for 30 min followed by reperfusion for 30 min resulted in a significant cardiac dysfunction and lipid peroxidation in saline-treated rat hearts. Further, ischemia-reperfusion was associated with a marked upregulation of β_1-AR density and protein and mRNA expression. Pretreatment of rats with atenolol preserved cardiac function, but did not affect the density of β_1-AR and the expression of β_1-AR protein and mRNA in the ischemic-reperfused myocardium. Pretreatment of rats with AS-ODNs directed at β_1-AR mRNA provided almost total preservation of cardiac function and lipid peroxidation following ischemia-reperfusion. AS-ODN treatment also prevented the upregulation of β_1-AR density, protein and mRNA expression in the ischemic-reperfused myocardium. The effects of AS-ODNs directed at β_1-AR mRNA on cardiac function were clearly superior to those of the commonly used β_1-AR blocker atenolol. The effects of AS-ODN on β_1-AR protein and mRNA expression indicate that its beneficial effects are mediated by inhibition of both transcription and translation of β_1-AR mRNA.

[0365] There is a generalized stimulation of the sympathetic nervous system during ischemia, perhaps a compensatory response designed to preserve cardiac dysfunction. Accordingly, catecholamine levels increase in both plasma and myocardium following myocardial ischemia (Rona, 1985; Richardt et al., 1994; Abrahamsson et al., 1983), but the increased catecholamine concentrations have the potential to contribute to increased excitability of myocardium resulting in arrhythmia (Strassere et al., 1990; Mukherjee et al., 1979; Maisel et al., 1985; Ohyanahi et al., 1988; Maisel et al., 1987). There is also increase in sensitivity of β_1-AR during acute myocardial ischemia (Strassere et al., 1990). This coupled with increased circulating and myocardial catecholamine concentrations can exacerbate cardiac injury and dysfunction.

[0366] It is generally accepted that activation of β_1-AR is the first element in the signal transduction chain mediating sympathetic stimulation of the heart. β_1-ARs, the dominant β-AR subtype in the heart (Minneman et al., 1995; Guder-

man et al., 1995), regulate cardiac function by activating adenylyl cyclase activity (Strassere et al., 1990; Thandroyen et al., 1986; Böhm, 1995). Several experimental studies have indicated an upregulation in β_1-ARs, but not β_2-ARs, during acute ischemia-reperfusion (Maisel et al., 1985; Karliner et al., 1989; Persad et al., 1998). Ihl-Vahl et al. (1995) conclusively demonstrated a rapid upregulation of β-AR mRNA during acute myocardial ischemia; this upregulation is subtype-selective with a specific increase mRNA level for β_1-ARs, but not for β_2-ARs. They also showed that the increase of β_1-AR mRNA is ischemia time-dependent.

[0367] Pretreatment of animals with β-AR blockers does not affect the ischemia-reperfusion-induced increase in β_1-AR mRNA level. This was confirmed in the present study wherein treatment with atenolol did not affect either the β_1-AR density measured by binding assays or the augmented β_1-AR protein and mRNA levels measured by Western analysis and RT-PCR, respectively. Other investigators have also shown that β_1-AR blockers do not block the expression of β_1-AR protein or mRNA (Aarons and Molinoff, 1982; Heilbrunn et al., 1986; Aarons et al., 1980). In contrast, the present study clearly demonstrates that AS-ODNs directed at β_1-AR mRNA block the up-regulation of β_1-AR number, and protein and mRNA levels. The blockade of β_1-AR at the transcriptional level may be the basis for the superior effect of the AS-ODN approach in the preservation of cardiac function after acute ischemia-reperfusion. Therapy with a single dose of AS-ODNs four days prior to removal of hearts prevented the increase in β_1-AR protein, which implies sustained inhibitory effects of AS-ODNs at translational levels as well. A previous study in the SHR indeed demonstrated that AS-ODNs against β_1-AR mRNA decreases β_1-AR mRNA translation into protein in the hearts for at least up to 18 days (Zhang et al., 1999).

[0368] Although chemical β-AR blockers are effective in the therapy of ischemic heart disease and are widely used in the short- and long-term management of patients with myocardial ischemia, these agents have several undesirable side-effects related to their effects on central nervous system, peripheral vascular resistance, and tracheo-bronchial tree. Even the selective β_1-AR blockers, such as atenolol, lose their cardio-selectivity at moderate doses. In addition, these agents need to be taken frequently, at least one daily, due to their short half-life. Furthermore, chemical βAR blockers do not influence β-ARs at genomic level. Gene therapy, such as AS-ODNs directed at β_1-AR mRNA, provides unique benefits. For example, the sequence specific AS-ODNs directed at β_1-AR mRNA can significantly decrease ventricular β_1-AR density by 30-50% even after 18 days in the rat after a single intravenous injection (Zhang et al., 1999). The AS-ODNs has no effect on β_2-AR density. Concurrently, AS-ODN administration is associated with reduction in blood pressure with a 38-mmHg maximum drop without causing bradycardia. Most importantly, there is no effect of AS-ODNs on the distribution of β-ARs in brain. These results demonstrate that a single intravenous injection of AS-ODNs can preserve cardiac dysfunction and protect myocardial injury from ischemia-reperfusion in the rat with specific effects on the expression of mRNA for β_1-ARs in myocardium. Importantly, IN-ODNs, used as control for AS-ODNs, did not show any of these effects.

[0369] In this study, ischemia-reperfusion-induced cardiac dysfunction was evaluated by the measurement of CPP, LVEDP, LVSP and dLVP. These indices of myocardial dysfunction have been used in several studies in the isolated heart model of global ischemia-reperfusion (Yang et al., 1998; Yang et al., 1993; Yang et al., 1997; Kokita et al., 1998; Ozden et al., 1998). Isolated rat, rabbit or guinea pig heart model provides an inexpensive and reproducible method to evaluate cardiac function and myocardial metabolic alterations during ischemia-reperfusion. This model has been used extensively to study regulation of variety of receptors and modulation of cardiac function by agents acting on different receptors (Lu et al., 1990; Yang et al., 1998; Neely and Rovetto, 1975; Baker and Pitha, 1982; Maisel et al., 1987; Yang et al., 1993; Yang et al., 1997; Kokita et al., 1998; Ozden et al., 1998). In the isolated beating heart, cardiac function can be assessed independent of the influence of circulating blood cells and hormones, which may be considered to provide an important advantage of this model.

[0370] MDA, a lipid peroxidation product, has been widely accepted as an index to evaluate myocardial injury after ischemia-reperfusion (Kokita et al., 1998; Ozden et al., 1998). All these parameters were modified by the use of AS-ODNs in the present study. In summary, the present study is the first report on the amelioration of cardiac dysfunction and myocardial injury induced by ischemia-reperfusion in isolated rat heart with a single intra-venous injection of AS-ODNs directed at β_1-AR mRNA. This study also provides evidence that the AS-ODNs can block the augmented expression of mRNA for β_1-AR in the ischemic myocardium. Lastly, the effects of AS-ODNs directed against β_1-AR mRNA appear to be superior to those of atenolol in the isolated rat heart model of ischemia-reperfusion.

5.5 Example 5

Reduction of Cardiac Hypertrophy in Animal Models

[0371] Adult spontaneously hypertensive rats (300-350 g) were treated with β1-AS-ODNs for more than 2 months. The blood pressure was reduced by 20-30 mmHg persistently throughout the treatment period (**FIG. 11**). Control animals receiving saline or INV-ODNs had no change in blood pressure. At the end of a 2-month treatment, the hearts were dissected and weighed. Left ventricular hypertrophy was indicated by the ratio of left ventricle weight to body weight (LV/BW). β_1-AS-ODNs significantly reduced left ventricular hypertrophy ($P<0.05$), compared to controls (**FIG. 11**).

5.6 Example 6

Attenuation of Cardiac Dysfunction After Myocardial Ischemia-Reperfusion

[0372] The protective effects of β1-AS-ODNs were tested in a rat model of myocardial ischemia-reperftision. Five groups of SD rats were studied. Sham controls (n=6) were injected with saline and perfused continuously for 80 min without ischemia/reperfusion (I/R). In other groups, hearts were perfused for 20 min and subjected to global ischemia (30 min) followed by reperfusion (30 min). Saline +I/R (n=6) or atenolol +I/R (n=7) groups were injected i.v. with saline or 2 mg/kg atenolol 6 hr before the hearts were excised. AS-ODN+I/R (n=7) or INV-ODN+I/R (n=7) were

injected iv. with 1 mg/kg β_1-AS-ODN or INV-ODN 4 days before the I/R study was performed.

[0373] Ischemia-reperfusion resulted in significant cardiac dysfunction, indicated by a significant increase in coronary perfusion pressure (CPP) and left ventricular end-diastolic pressure (LVEDP), and a decrease in developed left ventricular pressure (dLVP) ($P<0.01$ vs. pre-ischemia values).

[0374] Treatment with β_1-AS-ODN markedly attenuated the ischemia-reperfusion induced ventricular abnormality, manifested by the preservation of dLVP and minimization of increase in CPP and LVEDP ($P<0.05$ vs. saline group). Overall, AS-ODN treatment appeared to be equivalent to or even better than atenolol in these effects. AS-ODN restored dLVP to a greater extent than atenolol. INV-ODN showed no effect on ischemia-induced dysfunction and cannot be differentiated from saline group. Data on cardiac function parameters from these studies are summarized in **FIG. 12**.

5.7 Example 7

Safety Profile of Chronic Use of β_1-AS-ODNs

[0375] Safety profiles including liver transaminases, hematology, immune response and tissue pathology were investigated during three repeated injections of β_1-AS-ODNs for extended periods of time. The results indicated that long-term treatment with β_1-AS-ODNs is safe without causing toxic effects or immune reaction.

[0376] 5.7.1 Liver Transaminases

[0377] Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are routinely used as a clinical index for liver function. Their levels increase substantially upon liver damage. SHR were treated with three repeated injections of β_1-AS-ODN. Plasma concentrations of ALT and AST were measured after each injection, which revealed no significant difference between AS-ODN and control groups (**FIG. 13**).

[0378] 5.7.2 Hematology

[0379] Hematological parameters were measured after three repeated injections of β_1-AS-ODN. There was no change in total leukocyte count (WBC), platelet count, hematocrit and mean platelet volume (MPV) among three groups (Table 6).

TABLE 6

CLINICAL BIOCHEMICAL AND HEMATOLOGICAL PARAMETERS AFTER REPEATED ADMINISTRATION OF β_1-AS-ODN

	β_1-AS-ODN	INV-ODN	Saline
Liver Enzymes			
AST (unit/ml)	74.0 ± 3.7	83.0 ± 8.3	82.3 ± 3.8
ALT (unit/ml)	25.7 ± 1.1	30.5 ± 3.8	30.0 ± 3.2
Hematology			
hematocrit (%)	46.2 ± 0.63	46.0 ± 0.1	46.6 ± 0.33
WBC(10^3/mm^3)	5.48 ± 0.41	5.75 ± 1.15	5.86 ± 1.12
platelet (10^3/mm^3)	685 ± 12	706 ± 28	685 ± 22
MPV (fL)	6.93 ± 0.08	7.2 ± 0.20	7.0 ± 0.09
C-Reactive Protein	ND	ND	ND
Antibody to ODN	ND	ND	ND

n = 7–9 for each group,
ND: non-detectable.

[0380] 5.7.3 Immune Response

[0381] Antibody-antigen reaction was studied by gel immunodiffusion analysis, which revealed no detectable antibody against β_1-AS-ODN. C-reactive protein (CRP), a clinical index for immune reaction, was found negative in all animals.

[0382] 5.7.4 Tissue Histology

[0383] Heart, liver, spleen and kidney were subjected to pathological examination after three repeated injections of β_1-AS-ODN for more than 2 months. No immunopathology or organ damage was found.

5.8 Example 8

DNA Sequences of Known β_1-AR Genes

[0384] Particularly preferred antisense sequences for the practice of the present invention include those sequences that specifically bind at or near the region of the AUG translation initiation codon of the mRNA encoding a mammalian β_1-AR polypeptide. Thus, those complementary sequences that are centralized around the region of the mRNA corresponding immediately 5' of the AUG translation initiation codon are particularly preferred. Such sequences may be identified using a secondary structure analysis (e.g, the OLIGO computer program) to ensure that the oligonucleotides do not fold or self-anneal. The BLAST computer algorithm program may also be used to check the specificity of the chosen sequence. The ordinarily skilled artisan, having the benefits of the teachings of the present invention, can select other suitable antisense sequences using the OLIGO and BLAST programs in combination with the teachings of the subject invention to prepare both oligonucleotides that comprises at least 9 to about 35 contiguous nucleotides that are complementary to a portion of the mRNA encoding the open reading frames designated in each of the genes disclosed in SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, or SEQ ID NO: 194.

[0385] For the preparation of full-length or substantially full-length antisense polynucleotides, the inventors contemplate the use of sequences corresponding essentially to the entire β_1-AR-specific mRNA sequence produced from one or more of the following β_1-AR genes. For example, the open reading frame of the human β_1-AR gene extends from nucleotide 87 to nucleotide 1520 of SEQ ID NO:187. Therefore, a full-length antisense polynucleotide would consist of an approximately 1.43-knt (kilonucleotide) sequence

that is complementary to the region of SEQ ID NO: 187 from about position 87 to about position 1520. In order for the complementary sequence to specifically bind to the native β_1-AR-specific mRNA sequence, the polynucleotide need not be exactly the same size, or exactly the same sequence of the native mRNA. In fact, substantially full-length sequences or slightly larger-than full-length are contemplated by the inventors to be as useful in the inhibition of β_1-AR-specific mRNA translation in a host cell as full-length antisense polynucleotides. For example, the size of the complementary polynucleotide may vary (e.g., from about 95% to about 105% of the size of the ORF region of the particular β_1-AR-specific sequence. Thus, for a 1.43-knt full-length mRNA, a sequence of from about 1.36-knt to about 1.43-knt would be considered "substantially full-length" while a sequence of from about 1.44-knt up to and including about 1.50-knt would be considered "slightly larger than full-length," and complementary polynucleotides may be prepared in either of these size ranges that are sufficiently able to specifically bind to native β_1-AR-specific mRNA sequences in a host cell and inhibit the ability of the host cell's protein synthesis machinery to translate the native β_1-AR-specific mRNA sequence into functional β_1-AR polypeptide.

[**0386**] Such sequences, therefore, would be ideally suited for the preparation of the genetic constructs of the present invention, particularly in their exploitation as gene therapy vectors for the treatment of the cardiac diseases and disorders of the circulatory system described herein. In the preparation of such gene therapy vectors, one would preferably prepare a genetic construct in which a polynucleotide complementary to the mRNA was placed under the control of a suitable promoter, and then introduce the recombinant vector comprising the genetic construct into the host cells of the affected mammal. Expression of the encoded antisense polynucleotide, therefore, would result in the synthesis of a full-length, substantially full-length, or slightly larger-than full-length polynucleotide that is complementary to the native β_1-AR-specific mRNA produced by the cell. This complementary sequence could then specifically bind to the target mRNA and thereby reduce the availability of native β_1-AR-specific mRNA for translation into mature polypeptide by the cellular protein synthesis machinery.

[**0387**] For the human β_1-AR gene shown in SEQ ID NO:187, exemplary antisense polynucleotide sequences include those that are of from about 1.36-knt to about 1.50-knt in length and that comprise a substantially complementary contiguous sequence selected from about nucleotide 60 to about nucleotide 1600 of SEQ ID NO:187. The selection of substantially complementary, substantially full-length antisense polynucleotides capable of inhibiting the translation of native human β_1-AR mRNA into polypeptide is within the purview of the skilled artisan having the benefit of the present teaching and the selection methods, computer-based complementarity-defining search algorithms, and the assays for β_1-AR mRNA and β_1-AR polypeptides described herein.

[**0388**] In each of the following sequences, the AUG initiation codon is indicated as the first triplet in bold of the corresponding open reading frame.

[**0389**] 5.8.1 Human β_1-AR

[**0390**] The polynucleotide sequence encoding human β_1-AR polypeptide is disclosed in Frielle et al. (1987). The GenBank™ Accession No. for the *Homo sapiens* β_1-AR gene is NM000684. The Open Reading Frame extends from nucleotide 87 to nucleotide 1520 (shown in bold) (SEQ ID NO: 187)

```
  1 TGCTACCCGCGCCCGGGCTTCTGGGGTGTTCCCCAACCACGGCCCAGCCCTGCCACACCC
 61 CCCGCCCCCGGCCTCCGCAGCTCGGCATGGGCGCGGGGGTGCTCGTCCTGGGCGCCTCCG
121 AGCCCGGTAACCTGTCGTCGGCCGCACCGCTCCCCGACGGCGCGGCCACCGCGGCGCGGC
181 TGCTGGTGCCCGCGTCGCCGCCCGCCTCGTTGCTGCCTCCCGCCAGCGAAAGCCCCGAGC
241 CGCTGTCTCAGCAGTGGACAGCGGGCATGGGTCTGCTGATGGCGCTCATCGTGCTGCTCA
301 TCGTGGCGGGCAATGTGCTGGTGATCGTGGCCATCGCCAAGACGCCGCGGCTGCAGACGC
361 TCACCAACCTCTTCATCATGTCCCTGGCCAGCGCCGACCTGGTCATGGGGCTGCTGGTGG
421 TGCCGTTCGGGGCCACCATCGTGGTGTGGGGCCGCTGGGAGTACGGCTCCTTCTTCTGCG
481 AGCTGTGGACCTCAGTGGACGTGCTGTGCGTGACGGCCAGCATCGAGACCCTGTGTGTCA
541 TTGCCCTGGACCGCTACCTCGCCATCACCTCGCCCTTCCGCTACCAGAGCCTGCTGACGC
601 GCGCGCGGGCGCGGGGCCTCGTGTGCACCGTGTGGGCCATCTCGGCCCTGGTGTCCTTCC
661 TGCCCATCCTCATGCACTGGTGGCGGGCGGAGAGCGACGAGGCGCGCCGCTGCTACAACG
721 ACCCCAAGTGCTGCGACTTCGTCACCAACCGGGCCTACGCCATCGCCTCGTCCGTAGTCT
781 CCTTCTACGTGCCCCTGTGCATCATGGCCTTCGTGTACCTGCGGGTGTTCCGCGAGGCCC
841 AGAAGCAGGTGAAGAAGATCGACAGCTGCGAGCGCCGTTTCCTCGGCGGCCCAGCGCGGC
901 CGCCCTCGCCCTCGCCCTCGCCCGTCCCCGCGCCCGCGCCGCGCCCGGACCCCCGCGCC
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-continued

961 **CCGCCGCCGCCGCCGCCACCGCCCCGCTGGCCAACGGGCGTGCGGGTAAGCGGCGGCCCT**

1021 **CGCGCCTCGTGGCCCTACGCGAGCAGAAGGCGCTCAAGACGCTGGGCATCATCATGGGCG**

1081 **TCTTCACGCTCTGCTGGCTGCCCTTCTTCCTGGCCAACGTGGTGAAGGCCTTCCACCGCG**

1141 **AGCTGGTGCCCGACCGCCTCTTCGTCTTCTTCAACTGGCTGGGCTACGCCAACTCGGCCT**

1201 **TCAACCCCATCATCTACTGCCGCAGCCCCGACTTCCGCAAGGCCTTCCAGGGACTGCTCT**

1261 **GCTGCGCGCGCAGGGCTGCCCGCCGGCGCCACGCGACCCACGGAGACCGGCCGCGCGCCT**

1321 **CGGGCTGTCTGGCCCGGCCCGGACCCCCGCCATCGCCCGGGGCCGCCTCGGACGACGACG**

1381 **ACGACGATGTCGTCGGGGCCACGCCGCCCGCGCGCCTGCTGGAGCCCTGGGCCGGCTGCA**

1441 **ACGGCGGGGCGGCGGCGGACAGCGACTCGAGCCTGGACGAGCCGTGCCGCCCCGGCTTCG**

1501 **CCTCGGAATCCAAGGTGTAG**GGCCCGGCGCGGGGCGCGGACTCCGGGCACGGCTTCCCAG

1561 GGGAACGAGGAGATCTGTGTTTACTTAAGACCGATAGCAGGTGAACTCGAAGCCCACAAT

1621 CCTCGTCTGAATCATCCGAGGCAAAGAGAAAAGCCACGGACCGTTGCACAAAAAGGAAAG

1681 TTTGGGAAGGGATGGGAGAGTGGCTTGCTGATGTTCCTTGTTG

[**0391**] 5.8.2 Canine β_1-AR

[**0392**] The GenBank™ Accession No. for the *Canis familiaris* β_1-AR gene is U73207. The Open Reading Frame extends from nucleotide 330 to nucleotide 1751 (shown in bold) (SEQ ID NO: 188)

1 CCGGGTGCCGGGCCCGCGGGCTCGGCGCGCTCAGAAACATGCTGAGGTCCCGGCGGCTGT

61 TGCAGCAGCGGCAGCGGCTCCAGCAGCGGCTCCAGCAGCGGCTCCGGCAGCGGCTCCAGC

121 GGCGGCAACTCCGGCGGCAGCAGCAGCGGCGGCGGCGGCGGCGGCAGCGCACGGCTCCCG

181 CGGGGAAGGCGCCCGGCGCCCATGCCTCCGGCCCCGCGCGGCGGCTGCCCTGACCCGGCC

241 GCGACCTCCGCGCCCCACGTCCCCCGGCGTGGTCCCCGGCCACGGCCCCAGCCCGCCACA

301 CCCCCCGCCCCCGGCCTCCGCAGCTCGGC**ATGGGCGCGGGGGCGCTGGCCCTGGGCGCCT**

361 **CGGAGCCCTGCAACCTGTCGTCGGCCGCGCCGCTCCCCGACGGCGCGGCCACGGCGGCGA**

421 **GGCTGCTGGTGCCCGCGTCGCCGTCCGCCTCGCCGCTGGCCCCGACCAGCGAGGGCCCCG**

481 **CGCCGCTGTCGCAGCAGTGGACGGCGGGCATCGGGCTGCTGATGGCGCTCATCGTGCTGC**

541 **TCATCGTGGCGGGCAACGTGCTGGTGATCGCGGCCATCGCCAAGACGCCGCGGCTGCAGA**

601 **CGCTCACCAACCTGTTCATCATGTCCCTGGCCAGCGCCGACCTGGTCATGGGGCTGCTGG**

661 **TGGTGCCCTTCGGGGCCACGATCGTCATGCGGGGCCGCTGGGAGTACGGCTCCTTCCTGT**

721 **GCGAGCTCTGGACCTCGGTGGACGTGCTGTGCGTGACGGCCAGCATCGAGACCCTGTGTG**

781 **TCATCGCGCTGGACCGCTACCTGGCCATCACCGCGCCCTTCCGCTACCAGAGCCTGCTGA**

841 **CGCGCGCGCGCGCGCGGGCCCTCGTGTGCACCGTGTGGGCCATCTCGGCGCTCGTGTCCT**

901 **TCCTGCCCATCCTCATGCACTGGTGGCGGGCCGGGGGCGACGAGGCGCGCCGCTGCTACA**

961 **ACGACCCCAAGTGCTGCGACTTCGTCACCAACCGGGCCTACGCCATCGCCTCGTCCGTCG**

1021 **TCTCCTTCTACGTGCCCCTGTGCATCATGGCCTTCGTGTACCTGCGGGTGTTCCGCGAGG**

1081 **CGCAGAAGCAGGTGAAGAAGATCGACAGCTGCGAGCGCCGCTTCCTGGGCGGCCCCGCGC**

1141 **GGCCCCCCGCGCCCCCGCCCGCGCCCGCGCCCGCGCCCCCGCCCGCGCCCGGCTCCCCGC**

-continued

1201 **GCCCCGCCGCGGCCGCCCCGCTGGCCAACGGGCGCGTCGGCAGGCGGCGGCCCTCGCGCC**

1261 **TCGTGGCGCTGCGCGAGCAGAAGGCGCTCAAGACGCTGGGCATCATCATGGGCGTGTTCA**

1321 **CGCTGTGCTGGCTGCCCTTCTTCCTGGCCAACGTGGTCAAGGCCTTCCACCGCGACCTGG**

1381 **TGCCCGACCGCCTCTTCGTCTTCTTCAACTGGCTGGGCTACGCCAACTCGGCCTTCAACC**

1441 **CCATCATCTACTGCCGCAGCCCCGACTTCCGCAGGGCCTTCCAGCGCCTGCTGTGCTGCG**

1501 **CGCGCCGCGCCGCCCGCGGGAGCCACGGGGCCGCCGGGGACCCTCCGCGGGCCCGGCCGC**

1561 **CGCCGTCCCCCGGGGCCGCCTCGGACGACGACGACGACGACGAGGACGACGCCGGGGCCG**

1621 **GGGCCGGGGCCGCGCCGCCCGCGCGCCTGCTGGAGCCCTGGGCCGGCTGCAACGGCGGGG**

1681 **CGGCGGCCGACAGCGACTCCAGCCTGGACGGCGCGGGCAGCCCCGCGGGCGCCTCGGAGT**

1741 **CCCGGGTGTAG**GCGCGCGGCCTCCCGGGGGGCGCATCGGTGTTTACTCCAGACCCAGAGC

1801 AGGTGAACCCCGGGCCCGCGCACCCCCNTCGCATTCATCGAGGCA

[**0393**] 5.8.3 Sheep β_1-AR

[**0394**] The GenBank™ Accession No. for the *Ovis aries* β_1-AR gene is AF072433/S78499. The mRNA extends from nucleotide 1629 to nucleotide 4749 (underlined). The Open Reading Frame extends from nucleotide 2289 to 3692 (shown in bold) (SEQ ID NO:189)

1 TCCAGCCCCCTCTTTCTAGCCCTCTCCTTCCCTCATTTCCCCTTCTCAGGCTCCCCAACT

61 GGCAGAACTAAGCTGACAATCCTAAGCCAGGGATGCAGAAACAAGTAATTCACCCACATC

121 CACCCACTGATCATCAAGTTTGGGCCTAAAGCAAATTTACATGTTTGGATAAAGAAAAGT

181 TGGGCTTCCCTAGTAGCTGAGACCCATCTTCAGTCCTTGGATGGGGGAAGATCCCCTAGA

241 GAAGGAGATGGCAACCCACTCTAGTATTCTTGCCTGGAAAATCCCATAGGCAGAGGAGCC

301 TGGTGGCTACAGCCCATGGGGTTGCAAGAGTCAGACACAACTTAGCTACTAAAACCACCA

361 CCCATGGCTTATGAATACACATTGCTGTTAGCTCTCGACTTAGGGAGCTCTCTCCAAGGT

421 AAGAATATGAGTTTGTTCCTTTCAGAAACTATTCTTTTTATTCCAATGCTAGAAGGATGT

481 GTGAGCATTATGTAACATTTTCATGCACCCTTAAGTGGGTAATTAGAAGCTCTTTATTTC

541 TCAGGATTCAATTAAAAGCTTTTTATTTTCAAGGCTGAGTTGAGGACCAGTACTGTGGTG

601 GAATTAGACAAGGGGCTTGCACACCTTTGGCTACATTGTGTGTTGATGGGCCACCTTCCT

661 GTAGGTACCTCCCCACATATAGTCACACCACTGCAGAGCTAACGACTCACTAATTTTAAA

721 CCCATTCAGTTGCCAACCCAACAGCCTTTGATATAACTTTACATGCTATTGGATTTTAAT

781 CTTTTTGAGTATTTATATATGTTTTCTTCTCTCATCCCTCCAAAATTAATCCTAGAGTTT

841 TGAGAATCTGGGAACTTGGGCAAAGGAGAAGGCAACGCAGCAGACCAAGAAATTTGAAAT

901 CTCAGTTCACTACTGTGTCACCCAAAGTCAATGTACCTTTTTTGTTTGGACCGGCCCAGC

961 TCAAGTCATACAATCACGTGAGTAACAGACCACAAAATCCAGGTGTTATTACTGAACATG

1021 ACAAGTCTGAAAAGTAATTACACGTGTTCTAGCTTCCGTGGCGGTGTCATTTACTCTAAC

1081 ATGCCTGTCCTTAAGCCTCTCTCTCTCTTTACATTACCGGCACACACCGGTGCACCATAC

1141 TCACACATCCATCAGCTGGGACCTGGGAGTGTGTATTATTCCAACTGGTCCTCAGCATTA

1201 GCTGTCAGATGTCACAACCCCCYGCCGTTTTCTGCATCTGCTGCCCCGGGAAGCGAGAAG

1261 AAGCTTGCAAGAATAGCTCCCGGGAACGTTCCTGAAAGATTGGCGCTCTGCTTTAGCAAG

1321 GCGCTCGCTGGAAAGTTTCTTCTAACCGCTCACACCCGCCTCCGATCCGATCCCCGAGCT

-continued

1381 GGCAGGACGCGAGCTGGCTGGGACTCCTCTTGACAGAGGAAGGGCTTTACACACCACCCT

1441 CCTAGGCTGCCCAATACAAGAAACAGTCTTGCAGCCAGACTCCTCCACACCCAGCGAACA

1501 GACCGTCCAAGGCGCTCCGGTGTTTCGAGAACACCGAAGTCCCCTCCCTGCTAAAGGGCG

1561 CGTGAGCTCTGCTCTGCAGGAAACCTGGGCACTGGAGGTAGATGGGATGGGTGGCGGCGG

1621 GTAGAGCCGGGGCGCAGCGGAAAGCAAACGCCGGAGGCAAACGGGGCGCAGGAGAGGGGA

1681 GATTGGGTGCCGCCGTAGGGGCCAGGGTGAAAGCCGGGCGCGGACGGGAACCGAGGGGAA

1741 CTGGGCACTGGAGCCAAGCGGGCTCTGGAAGGGACGCGCGGGCAGGAACCCGCGAGCGCT

1801 GGGGAGGGGCTTGCTTGGCGATCTGCCCCGGACTCCCTAGAGCCGCAGAACCGCCGGTGG

1861 AGGCGGGGTGCTAGGAGTTGGCGGGGCCGGGTGGGGGTGGGGGGGAACCAGAGAGGGGCG

1921 TGCCTTCGCCAGGATTGGCTGCAGGAGCCTGACGCGAGNNNCCGGGGGTTGGCTCGGGGG

1981 AGTGGGAGCCGGGTGGGGTGGGTGCTGGGTGCCGGGGCTGCGGGCTCCGCGAGCTCAGAA

2041 ACATGCTGAGGTCCCGGCAGCTGTTCCAGCAGCGACACCACTCCAGCAGCAGCCGCGGCG

2101 GCTGCGGCGGCGACAGGCACCGGCTCCGGCGGGGAAGGCGCCCGGCGCCATGCCTCCGGC

2161 CCCGCGCCGCGCTGCGCTGACCTGGCCGCGACCTCCCTCCGCGCGCCCCGCCGTTCGGGC

2221 CTCTGGGGGGTTCCCCAACCGCGGCCCAACTCCGCCACACCCCTCTCCCCCGGCCTCCGC

2281 AGCTCGGC**ATGGGCGCGGGGGCGCTCGCCCTGGGCGCCTCCGAGCCCTGCAACCTGTCAT**

2341 **TCGCCGCGCCGGTCCCCGACGGCGCGGCCACGGCGGCGGCGCGGCTGCTAGTTCCCNCGTCGC**

2401 **CGCTCCGCCTCGCTGCTGACCTCGGCCAGCGAGGGACCCCGCTGCTGTCGCAGCAGTGGA**

2461 **CGGTCGGCATGGGCCTGCTGATGGCATTCATTGTGCTGCTCATCGTGGCGGGCAACGTGC**

2521 **TGGTGATCGTGGCCATCGCCAAGACTCCGCGGCTGCAGACGCTCACCAACCTCTTCATCA**

2581 **TGTCGCTGGCCAGCGCAGACCTGGTCATGGGTCTGCTGGTAGTGCCGTTTGGAGCCACAA**

2641 **TCGTGGTGTGGGGCCGCTGGGAGTATGGCTCCTTCTTCTGCGAGCTCTGGACCTCGGTGG**

2701 **ACGTGCTGTGCGTGACGGCCAGCATCGAGACCCTGTGTGTCATCGCCCTGGACCGCTACC**

2761 **TCGCCATCACGTCGCCCTTCCGCTACCAGAGCCTGCTGACCCGCGCGCGAGCGCGGGCCC**

2821 **TCGTGTGCACCGTGTGGGCCATCTCGGCGCTGGTGTCCTTCCTGCCCATCTTCATGCAGT**

2881 **GGTGGGGGGACAAGGACGCCAAGGCGAGCCGGTGCTACAACGACCCCGAGTGCTGCGACT**

2941 **TCATCATCAACGAGGGCTACGCGATCACCTCTTCCGTCGTCTCCTTCTACGTGCCCCTGT**

3001 **GCATCATGGCCTTCGTGTACCTGCGGGTGTTCCGCGAGGCCCAGAAGCAGGTGAAGAAGA**

3061 **TCGACAGCTGCGAGCGCCGCTTCCTCAGCGGCCCCGCGCGGCTGCCCTCGCCCGCGCTCT**

3121 **CGCCCGGGGCGCCGCTCCCTGCCGCCGCGGTGGCCAACGGGCGCGCCAACAAGCGGCGGC**

3181 **CCTCGCGCCTCGTGGCCCTGCGCGAGCAAAAGGCCCTCAAGACGCTGGGCATCATCATGG**

3241 **GCGTGTTCACGCTCTGCTGGCTGCCCTTCTTCCTGGCCAACGTGGTGAAGGCCTTCCACC**

3301 **GCGACCTGGTGCCCGACCGCCTCTTCGTCTTCTTCAACTGGCTGGGCTACGCCAACTCGG**

3361 **CCTTCAACCCCATCATCTACTGCCGCAGCCCCGACTTCCGCAAGGCCTTCCAGCGCCTGC**

3421 **TCTGCTGCGCGCGCCGGGCCGCCTGCGGGAGCCACGGGGCCGCCGGGGACCCGCCGCGCG**

3481 **CCGCGGGCTGCCTGGCGGTGGCCCGGCCGTCGCCGTCTCCCGGGGCCGCCTCGGACGACG**

3541 **ACGACGACGACGACGAAGACGACGTCGGGGCCGCGCCGCCCGTGCGCCTGCTGCAGCCCT**

3601 **GGGCTGGCTACAACGGCGGGGCGGCGGCGAACAGCGACTCGAGCCCGGACGAGCCAAGCC**

-continued

3661 **GCCCGGGCTGCGGCTCGGAATCCAAGGTGTAGGGACGGGCGCCCCTCCCCGCCTTCCCCG**

3721 **GCTTCCCCAGTCCGGGAGCGGGCTGTGCGCTCCAGGAGCAAGAGAACCCGGGCGMCCCTG**

3781 **AACCGCTTCCCCGGGAAAGAGGTCTGTGTTTACTCGAGACCGTAAAGCAGGTGAACTCGA**

3841 **AGCCTGCGAACCTCGTCTGCATCATCCAAGGGCAAATAGGAAAGCCACGGACCGTCGCAC**

3901 **AGAAAGGAAAGTTTGGGGAGAGGTGGGAGAAGTTTGGGGAGGGGTGGAAGAGTGGCTTAC**

3961 **TGATTGTTCTTGGGGTTCTTTTTCCTGTTTCT**GGTCCAGCCTTCTGTGTGTGCGTGTGAT

4021 GCATCTTTAGAGTCCCTCCTCCCCCCGCCCCCCCGCGACGTGGCTTTTAACACTTTCTGC

4081 GATGACTGGGAAGGGAAGGGGGAAGCGTTAGGAGGTAAAAGTCTCTCGACTTAGTTTCCA

4141 TCCCATTCCTGGGAACAGAAGCGGTCAGCCAGAGAGAGAGGAGAGAGAATGACACTTTAT

4201 CAGGACGTTGTTTCCTTTTGCTTTTCAGAGAAATACCATTTTAATTTCTGAGGAATTATT

4261 TCTCCTGTTCTGAAAGCCGAGGGCAAGGATGGATGCAAAAATCGCGTTTCAGGAAGTTTT

4321 ATGCTCTTCTTGGAACAAGCCTCACCTTGCTTCCCTTTCGGAGGGCAAGCGGGCTGTCCC

4381 TGAACGCCTCCTCGGTGGTCAGGCTGAGGGGTTCCTACCTCACTCACACGGTGCACATTG

4441 CACGGCCAGATAGAGAGACTTGTTTATATTAAACAGCTTATTTATGTATCAATACTAGTT

4501 GGAAGGACCAGGCGCTGAGCCTTCGTGACATGTGACTCTGTCCATTGAAGACAGGACAGA

4561 AAAAGGAAAAAGAAAAAAGGAAAACAATTCAGATTACTGCACATGTGGTATAGACAAAAA

4621 ATCAAAACAAAAAAGCCGTGATTCAAAGTGGCATTTTTTTTGCACAGTATTAGGAACTGT

4681 AAAGTCCACAGAAAATGTTATTTGCACAAAAAGAAATGAAATATTTTTTAATGGGAGTGG

4741 GGTGGGGCA

[**0395**] 5.8.4 Porcine β_1-AR

[**0396**] The GenBank™ Accession No. for the *Sus scroffa* β_1-AR gene is AF042454. The Open Reading Frame extends from nucleotide 456 to nucleotide 1862 (shown in bold) (SEQ ID NO:190)

1 GACTCCCCAGAGCTGCTGAACCGCCGGGGGGAGGGGGTGGGGGAGTTGGCGGGGGGG

61 GGGGGGGAGAACCAGATCGGGGCGTGCTTCCTCGGATTGCTGCAATAGCTGACGCGA

121 GGCCCCGGGGGTTGGCTCCAGGGAGTGGGATGGGAGCGGGTGGGGTGGTGCTGGGTG

181 CCGGGGCTGCGGGTTCCGGCGCTCAGAAACTGCTGAGGTCGCAGCTGTCCAGCAGCG

241 ACACCGCTCCAGCAGCAGCGGCGGCGGCGGGGCGGCGACGCGCACAGCCTGGCGGGG

301 AAGGCGCCCTGCGCCCATGCCTCCGGCCCCCGCCGCGGCGCCCTGACCGGCCGCGAC

361 CTCCCTCGTCGCGCCCCGCCGCCCGGGCCTTGGGGGGGTCCCGACCGCGCCCAACTC

421 CGCCACACCCCCCGCCCCCGCCTCCGCAGCCGGT**ATGGGGCGGGGGCGTCGCCCTGG**

481 **GTGCCTCCGAGCCCTGCAACCTGTCATCGGCGCGCCGCTCCCGACGGCCGGCCACCG**

541 **CGGCGCGGCTGCTGGTGCCTGCGTCCCCTCCGCCTCGCTCTGACCCCACCAGCGAGG**

601 **GATCCGTGCAGCTGTCGCAGCAGTGGACGGTGGCATGGGCTCCTGATGCGCTCATCG**

661 **TGCTGCTCATCGTGGCGGGCAACGTGCTGGGATCGTGGCATCGCCAAGCGCCGAGGC**

721 **TGCAGACGCTCACCAACCTCTTCATCATGTCCTGGCCAGGCCGACCTGTCATGGGGC**

781 **TGCTGGTGGTGCCATTCGGGGCCACCATCGGGTGTGGGGCGCTGGGAGACGGCTCCT**

-continued

841 **TCTTCTGCGAGCTCTGGACGTCGGTGGACGACTGTGCGTACGGCCAGTTCGAGACCC**

901 **TGTGTGTCATCGCCCTGGACCGCTACCTCGCATCACGTCCCCTTTCGCACCAGAGCC**

961 **TGCTGACCCGCGCGGCACGGGCCCTCGTGTCACCGTGTGGCCATCTCGCCCTGGTGT**

1021 **CCTTCCTGCCCATCCTCATGCACTGGTGGCGGACAAGGGGCCGAGGCAGCCGCTGCT**

1081 **ACAACGACCCCAAGTGCTGCGATTTCGTCACAACAGGGCTACGCCATCCCTCGTCCG**

1141 **TGGTCTCCTTCTACGTGCCCTTGTGCATCAGGCCTTCGTTACCTGCGGTGTTCCGCG**

1201 **AGGCCCAGAAGCAGGTGAAGAAGATCGACACTGCGAGCGCGCTTTCTCGCAGCCCCG**

1261 **CGCGGCCGCCCTCGCCCGCGCCCTCGCCCGGTCCCCGCTCCTGCCGCCCTGCCGCAG**

1321 **CCCCGGTAGCCAACGGGCGCACCAGCAAGAGCGGCCCTCCGCCTCGTGCCCTGCGAG**

1381 **AGCAGAAGGCGCTCAAGACGCTGGGCATCACATGGGCGTTTCACGCTCGCTGGCTAC**

1441 **CCTTCTTCCTGGCCAACGTGGTGAAGGCCTCCACCGCGACTGGTGCCCACCGCCTCT**

1501 **TCGTCTTCTTCAACTGGCTGGGCTACGCCACTCGGCCTTAACCCCATCTCTACTGCC**

1561 **GCAGCCCTGACTTCCGCAAGGCCTTCCAGCCCTGCTCTGTGCGCGCGCGGGTCGCCC**

1621 **GCGGGAGCTGCGCGGCCGCCGGGGATGGGCGCGCGCCTCGGCTGCCTGCGGTGGCCC**

1681 **GGCCGCCGCCGTCGCCCGGGGCCGCCTCGGCGACGACGAGACGAAGAAACGTCGGGG**

1741 **CCGCGCCGCCGGCGCCCCTGCTGGAGCCCTGGCCGGATAAACGGCGGGCGGCACGTG**

1801 **ACAGCGACTCGAGCCTGGACGAGCGGACGCCGGGGGCCGGCCTCGGAACCAAGGTGT**

1861 **AG**GGCCCAGCGCTCCCTCCCCACCTCCCAGGGGGATGCAGCTCTGCGCGAGAGAAGA

1921 GAACCCGGCGCCCCGAAAGGCTTCCCGGGGATGAGGAGACTGTGTTTATCGAGACCG

1981 AAAGCAGGTGAACTCGCAAACCTCGTCTGCTCATCTAAGCAAACAGAAAGCCGGACC

2041 GCTGCACAGAAAGGAAAGTTTGGGGAGGGTGGAGAGTGGTTGCTCATTTTCTTGAGT

2101 TCTTTTCTCTGTTTGTGGTCCGTCCTCCTTTGTGTGTGCTGTGATGCACTTTAGATT

2161 TTTATTCCCCCAGGTGGTTTTTAACACTTTTGCGAAGACAGGGAAGGGTGGGAGAAG

2221 CAGGAGAGTTTTTAAAAAGGTGTCTCAACTGGCTTCCATCCGTTCCCGGGACGGAGC

2281 AGTC

[**0397**] 5.8.5 Rat β_1-AR

[**0398**] The rat β_1-AR polypeptide and polynucleotide sequences are disclosed in Machida et al. (1990). The GenBank™ Accession No. for the Rattus norvegicus β_1-AR gene is D00634. The Open Reading Frame extends from nucleotide 1257 to nucleotide 2657 (shown in bold) (SEQ ID NO:191)

1 GGTACCAGAGTACAAACGTCGGTGTTAAGGTGTTGGTACTGGAGTCAAAAGTCTGAAG

61 CGTCATTCTCTGAGTTCTTTTACGCGGGGTGGGGGTTGGGGGTGGGGCGTGTCATTTA

121 CTCCGGACTTAATGTTGCCCAAACTTCTCCTTTATACTCAGAACTCATCCATCCTTTT

181 CCTTAGCTGGGATTGGGAACGTGTGTGTCCAACTGGTCCTTGGCGTTGGAGTTACAGC

241 CACAAACCTTCCCTTCCTTCCCCATCCACTGCACTTGAGGGAGAGAGAGACAGCTCTG

301 CGGAAGAACAGCGCCCAGAGGCCTTTCTGACAGATGGCACTCTGCTCCCCAATCCGCC

361 TGGCTGCAAAGTTTCCTCTATAACTAACACCCACTTCCTATTCCCCAAGTGTCATTCT

421 GGCCGGGTCCCCTCGCGATCGGGAAGAGCCAGGCTGCCTGATGGCAGAACAGTCTCAC

-continued

481 AGCTAAATTCTCTAATCCCACAGATAGGCTGTCCAAGGCATCTCCGGAAGCCAGCAAG

541 TTTCCCTTCTAGCTACCAGGACCATGCACTAGAGGTAGAAAGGCGAGGGGCTACGGGG

601 CGCCGAAGGGAAACCGGCTCTGCGGGAGGGCAAAATGAGAGCTGGGTGCAGGGCAAGC

661 GGACACCACTTGGGCGGTGGGGTGGGCACAGGAGGCCGGCGGGGCACCCAGAGGGCGC

721 AGCAGACCAGACTCTGGAAGAGCCTGAGCAGGAAAGGGCGCGCTCGCTGAGCCCGTGG

781 GTGCCGGCTGCGTAGTCCACCGCAATCTTTGGAGCCTTTCAATCGCGGTGGAGGGGGT

841 GCTGGGTGTTGGGGGTGGGGCACCATTGTTCGGGGGCGTGCCTTGGACGCGATTGGCT

901 GCGGGAGCCTGACGCGCGGCCCGGGGGCTGGCTGGGGGGTAGGGAGCGAGTGGGGGGG

961 AGGTGCTGGGTGTTGGAGCCCCGGCCCCGCGCGCTCAGAAACATGCTGAGTCCCGGCA

1021 ACTCTTCCAGCAGCGACCCGTCCAGCAGCAGCGGCGGCGGCGGCGGGACACGGCT

1081 TGGCTACGGAGGAGAAGGCGCCGGCCTCCATGCCTCCGGCCCCAAGCCGGGCTGCCCT

1141 GACCTGGCCGCGACCTCCCCCGTCCCCGCGCGCGCCCCCAGCCCCGGCTCTGGGGTGC

1201 TTCCCAGGCGCGGCCCAGTCCGCCACACCCCCCGCGCCCGGCCTCCGAATCGGC**ATGG**

1261 **GCGCGGGGGCGCTCGCCCTGGCGCCTCCGAACCCTGCAACCTGTCGTCGCCGCGCCGC**

1321 **TGCCCGACGGCGCGGCCACCCGGCACGACTGCTGGTGCTCGCGTCGCCTCCGCCTCGC**

1381 **TGCTGCCTCCAGCCAGCGAGGCTCAGCGCCGCTGTCGCAGCAGTGGACCCGGGTATGG**

1441 **GCCTACTCCTGGCGCTCATCTGCTGCTCATCGTAGTGGGCAACGTGTTGTGATCGTGG**

1501 **CCATCGCCAAGACCCCGCGGTGCAGACGCTCACCAACCTCTTCATCATGCCCTGGCCA**

1561 **GCGCCGATCTGGTCATGGGATGCTGGTGGTGCCTTTCGGGGCCACCATTTGGTGTGGG**

1621 **GCCGCTGGGAGTACGGCTCCTCTTCTGTGAGCTCTGGACTTCGGTAGACTGCTATGTG**

1681 **TGACGGCCAGCATCGAGACCTGTGTGTCATCGCCCTGGACCGCTACCTCCCATCACGT**

1741 **CGCCCTTTCGCTACCAGAGCTGCTGACGCGCGCGCGAGCGCGGGCCCTCTGTGCACAG**

1801 **TGTGGGCCATCTCCGCGCTGTGTCCTTCCTGCCCATCCTCATGCACTGGGGCGGGCCG**

1861 **AGAGCGACGAAGCGCGCCGCGCTACAACGACCCCAAGTGCTGCGATTTCTCACCAACC**

1921 **GGGCCTACGCCATCGCCTCGCCGTCGTCTCCTTCTACGTGCCCCTGTGCTCATGGCCT**

1981 **TCGTGTACCTCCGGGTGTTCGCGAGGCCCAGAAACAGGTGAAGAAGATCACAGCTGCG**

2041 **AGCGCCGCTTCCTCAGCGGCCGCCCCGGCCGCCCTCGCCCGCGCCCTCGCATCACCAG**

2101 **GGCCACCGCGCCCCGCAGACCGCTGGCCAACGGGCGCTCCAGCAAGCGGGGCCGTCGC**

2161 **GCCTCGTGGCTCTGCGAGAGAGAAGGCGCTCAAGACACTGGGCATCATCTGGGTGTGT**

2221 **TCACGCTCTGCTGGCTGCCCTCTTCCTGGCCAACGTGGTGAAAGCTTTCACCGCGACC**

2281 **TGGTGCCGGATCGCCTCTTCTCTTCTTCAACTGGCTGGGCTACGCCAACCGGCCTTCA**

2341 **ACCCCATCATCTACTGCCGCGCCCCGACTTCCGCAAGGCTTTCCAGCGCTGCTTTGCT**

2401 **GCGCGCGCCGGGCCGCCTGCGACGCCGCGCAGCCCACGGGGACCGGCCGGCGCCTCGG**

2461 **GCTGCCTGGCGAGAGCTGGGCGCCGCCGTCCCCCGGGGCTCCTTCGGACACGACGACG**

2521 **ATGACGCCGGGGCCACCCCACCGCGCGCCTGTTGGAGCCCTGGGCCGGCGCAACGGCG**

2581 **GGACGACCACTGTGGACAGCATTCGAGCCTGGACGAGCCGGGACGCCAGGCTTCTCCT**

2641 **CCGAGTCCAAGGTGTAG**AGGCCAGGCTCTCCGGGCGCACGGACGCCGCTCCCATAGTC

2701 CCGGGCTGGACACGGGCTCTCATCCCTAGAGGAAGAGAGCCCGCCTGGGCCCTGAGCC

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2761 GCTCCCCAGGGGAGAGAGGAATTTCTGTTTACTCAAGACCGAAAGCAGGGAATGCGAA

2821 GCCCACAGATCTTTTGAATCTCCGAGACGTACAGAAAAGCCCCGGACCGGCGTTGCGC

2881 AAAAAGGAAAGTTTGGGAGTGTTGGGAGAGTGTGGCTTAGTGTGGCTTATGGCTTGTC

2941 TTGAGTTCCTTTCTCTCTGAGTTCGGCCTTTCGTGTGTTTAATGCACCTTAGGCACCC

3001 CCCCCCCGTGGGTTTTGACATTTCTGCAAGGACCCGAGTGGAAACTAGGGGGGAGGGG

3061 AAGGGGGAGGGTGGAGTCTACACCTGCCTTCTACTTCACACCTAGGAACTAAGTGTTC

3121 AGCTCTGGTTTGGGGTGGGCCAGGAGCGGTGATAATTAGCCAGGAAGTGTCCTTTTGC

3181 TTTCTAAAGAAATTGCATATAATTCCGGAGTATTGGTGTCTCCTTAAAGAAAGGGGGG

3241 AAAGGTGGCTGAGAAACAGACAATCTGGTTTCGAGAAACTATTTGTGGACACGGTTCA

3301 CCTTGCTTTCTCCTGGAGGGAAACCCTGTCCCTGCGCGCCTCGGTGGTCTGCTGTGGG

3361 TCCTCTACCTCACTCTGTGCTATTGCACAGCAAGATAGAAAGACTTGTTATATTAAAC

3421 AGCTTATTTATGTATCAATATAGTTGGAAGGACCAGGCGCTGAGTCTCTTCTGTGACA

3481 TGTGATTCTGTCAACTAAAGTAGGACAGAACAAAAGGAAACAGTTGGGATATTGCACA

3541 TGTGGCTAAAAACAAAGATGCAAAAAAAAAAGGCAGTGGTTGAAAGGCCTTTTGCGCA

3601 GTGTTAGACATTGTAAAATCATAGAAGTTGTTAACTTGCACAAAAAATTAATATTTTT

3661 AATGGGACGGGGAGGTGGGCGATCT
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[0399] 5.8.5 Rhesus Monkey β_1-AR

[0400] The GenBank™ Accession No. for the *Macaca mulatta* β_1-AR gene is X75540. The Open Reading Frame extends from nucleotide 1425 to nucleotide 2867 (shown in bold) (SEQ ID NO:192)

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   1 CCAAGGAATCTGAAATCGCAGTTCATGACGTCAGCATAAACCGACAGTACCTTTTTGTTT

  61 GTATTGACCCAGCTCAAATTATAAAATCACATGAGTAATAAAACACAAAATACAGGTGTT

 121 GTCACTGAAAAGCCTAAAATGTAACTAAACGCGTTTTCCCCCCTCGCGGTGGTATCATTT

 181 ACTCTGTATCTCAACGTTTCTGTCTCCCTAAGACTCTCCCTTTATATCGTGAGCACACAT

 241 TTTTGCATCATGCTCACACATTCATTAGCTAGGACTGAGGAGTGTGTACGATTCCAACGG

 301 GCCCTCAGCGTTAGCTGTTAGATGCACAAACCTTCACTTCCTTTCCACATCTACTGCACT

 361 TGAGGTTCAACAGAGGATCCTTGCAAGAACAGTGCCCAGAGAGCATTCTTGACAGATGCG

 421 CGCTTAGCTCCAGCAACCCGCTCTGCTGGGAAGTTTCTTCTAACCACTAACACCCACCTC

 481 CAATCCCCCAAGCTGTCACGACGCAGGCTGGCTGGGTCCCGTCTTGACGGGGGAAGGGTT

 541 TTACACACATCCTGCTAGGCTGCCCCACATCACAACCAAGCTCGCAGGGCAAACTCCTCC

 601 AAGCCTGGCGGACAAGCTGTCCCAGGCGCTCTGGCGCTTCCTGAACACCAAGGTCCCCTC

 661 CGCGCTCAAGGGAGCTAGCGCACTGTTACGCAGGGAACCCCGGCACTGCAGGTCGAGGGG

 721 ATGCCGAGGGAGCGGGGGCGCAGCAGGCAGCCGACTGCTGTAGGCAAACGGCGCGCAGGA

 781 GGCAAACGAGGCGCAGGAGCCGGTGCGAGAGCGAGTGGGCGCTGAGAGAGGGGGCGGGGC

 841 CCCAGGGGGGAGGCGAGCGCGGGAGGGGGCACGGGAGAACAGGGACCAGGAACCAGCGGG

 901 CGCAGGAAGGGGTGCGTCCGCAAGAACCCGCGGGCGCACGGGAGGCACTAGCTCCACGAT

 961 CAGCTCGGGACTCTCAGGAGCCGCTCAATTGCCAACGGGAGGGGGCTGCGGGGAGTTGGA
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1021 GGTTGGGGGGGCTGACCAGACGGGGGCGTGCCTTTGCCCGGATTGGCTGCAGGAGGCTGA

1081 CGCGAGGCCCCGGGGGTTGGCTTGGGGAGTGGGAGCGGGGTGGGGTGGGTGCTGGGTGCC

1141 GGAGCTGCGGGCCCGGCGCGCTCAGAAACATGCTGAGGTCCCGGCGGCTCTTCCAGCAGC

1201 GGCAGCGGCTCCAGCAGCAGCGGCGGCGGCGGCGGCGGCAGCGACAGCGCTCGGCTC

1261 CGGCGGGAAAGGCGCCCGGCGCCCATGCCTCCGGCCCCGGGCCGCGGCTGCCCTGACCCG

1321 GCCGCGACCTCCCTCTGCGCACCCCGCCGTCCAGGCTTCTGGGGTGTTCCCCAACCAAGG

1381 CCCAGCCCTGCCACACCCCCCGCCTCCGGCCTCCGCAACTCGGC**ATGGGCGCGGGGGCGC**

1441 **TCGTCCTGGGCGCCTCCGAGCCCGGTAACCTGTCGTCGGCCGCACCGCTCCCCGACGGCG**

1501 **TGGCCACCGCGGCGCGGCTGCTGGTGCCCGCGTCGCCGCCCGCCTCGTTGCTGCCTCCCG**

1561 **CCAGCGAAGGCCCCGAGCCGCTGTCGCAGCAGTGGACGGCGGGCATGGGTCTGCTGATGG**

1621 **CGCTCATCGTGCTGCTCATCGTGGCGGGCAACGTGCTGGTGATCGTGGCCATCGCCAAGA**

1681 **CGCCGCGGCTGCAGACGCTCACCAACCTCTTCATCATGTCCCTGGCCAGCGCCGACCTGG**

1741 **TCATGGGGCTGCTGGTGGTGCCGTTCGGGGCCACCATCGTGGTGTGGGGCCGCTGGGAGT**

1801 **ACGGCTCCTTCTTCTGCGAGCTGTGGACCTCGGTGGACGTGCTGTGCGTGACGGCCAGCA**

1861 **TCGAGACCCTGTGTGTCATCGCCCTGGACCGCTACCTCGCCATCACCTCGCCCTTCCGCT**

1921 **ACCAGAGCCTGCTGACGCGCGCGCGGGCGCGGGGCCTCGTGTGCACCGTGTGGGCCATCT**

1981 **CAGCCCTGGTGTCCTTCCTGCCCATCCTCATGCATTGGTGGCGGGCGGAGAGCGACGAGG**

2041 **CGCGCCGCTGCTACAACGACCCCAAGTGCTGCGATTTCGTCACCAACCGGGCCTACGCCA**

2101 **TCGCCTCGTCCGTGGTCTCCTTCTACGTGCCCCTGTGCATCATGGCCTTCGTGTACCTGC**

2161 **GGGTGTTCCGCGAGGCCCAGAAGCAGGTGAAGAAGATCGACAGCTGCGAGCGCCGTTTCC**

2221 **TCGGCGGCCCCGCGCGGCCGCCCTCGCCCTCGCCCTCGCCCTCGCCCTCGCCGGTCCCCG**

2281 **CGCCGCCGCCCGGACCTCCGCGCCCCGCCGCCGCCGCTGCCACCACCGCCCCGCTGGTCA**

2341 **ACGGACGTGCGGGTAAGCGGCGGCCCTCGCGCCTCGTGGCCCTGCGCGAGCAGAAGGCGC**

2401 **TCAAGACGCTGGGCATCATCATGGGCGTGTTCACGCTCTGTTGGCTGCCCTTCTTCCTGG**

2461 **CCAACGTGGTGAAGGCCTTCCACCGCGAGCTGGTGCCCGACCGCCTCTTCGTCTTCTTCA**

2521 **ACTGGCTGGGCTACGCCAACTCGGCCTTCAACCCCATCATCTACTGCCGCAGCCCCGACT**

2581 **TCCGCAACGCCTTCCAGCGACTGCTCTGCTGCGCGCGCAGGGCTGCCCGCCGGCGCCACG**

2641 **CGGCCCACGGAGACCGGCCGCGCGCCTCGGGCTGTCTGGCCCGGCCCGGACCCCCGCCGT**

2701 **CGCCCGGGGCCGCCTCGGACGACGACGACGACGATGTCGTCGGGGCCACGCAGCCCGCGC**

2761 **GCCTGCTGGAGCCCTGGGCCGGCTGCAACGGCGGGGCGGCGGCGGACAGCGACTCGAGCC**

2821 **TGGACGAGCCGTGCCGCCCCGGATTCGCCTCGGAGTCCAAGGTGTAG**GGCCCGGCGCCGG

2881 GCGAGGACGCCGGGCACCCCGGGAGGAGGAGAGCCCGGGCGCCCCGGAACGACTTCCCGG

2941 GGGAACGAGGAGATCTGTGTTTACTCAAGACCGAAAGCAGGTGAACTCGAAGCCCACAAT

3001 CCTCGTCTGAATCATCCGAGGCAAACAGAAAAGCCACGGACCATTGCACAAAAAGGAAAG

3061 TTTGGGGAGGGATGGGAGAGTGGCTTGCTGATGTTCCTTGGTTTTTTTTCTTTCTTTCTT

3121 CCTTTTTTTTTTTTTTTTTCTGTTTGTGGTCCGGCCTTCTTTTGTATGTGCGTGTGATGC

3181 ATCTTTAGATTTTTTTTCCCCCACGTTGGTTTTTGACACTCTCTGCGAGGACCGGAGTGG

3241 AAGTTGGGTGGGTTACGGGAAGGAAGAAGCATTAGGAGGGGATTAAAATCGATCAGCGTG

-continued

3301 GCTCCTATCCCTTTCCCAGGAACAGGAGCAGTCTACCAGCCAGAGGGAGGAGAATGACAG

3361 TTTGTCAAGACGTATTTCTTTTGCTTTCCAGATAAATTTCATTTTAATTTCTAAGTAATG

3421 AGTTCTGCTGTCATGAAAGCAAAGAGAAAGGATGGAGGCAAAAAAAAAAAAATTCACGTT

3481 TCAAGAAATGTTAAGCTCTTCTTGGAACAAGCCCCACCTTGCTTTCCTTGTGTAGGGCAA

3541 ACCGGCCGTCCCCCGCGCGCCTGGGTGGTCAGGCTGAGGGATTTCTACCTCACACTGTGC

3601 GTTTGCACAGCAGATAGAACGACTTGTTTATATTAAACAGCTTATTTATGTATCAATATT

3661 AGTTGGAAGGACCAGGCGCAGAGCCTCTCTCTGTGACATGTGACTCTGTCAATTGAAGAC

3721 AGGACATTAGAGAGAGAGAAACAGTTCAGATTACTGCACATGTGGATAAAAACAAAAACA

3781 ACAAAAAAAGGAGTGGTTCAAAATGCCATTTTTGCACAGTGTTAGGAATTACAAAGTCCA

3841 CAGGAGATGTTACTTGCACAAAAAGAAATTAAATATTTTTTAAAGGGGGAGGGGCTGGGC

3901 AGATCTTAAAAACTAAAATAAAATTCAAACTCTACTTCTGTTGTCTAGTATGTTATTGAG

3961 CTAATGATTTATTGGGGAAAATACCTTTTTATACTCCTTTATCATGGTACTGTAACTGTA

4021 TCCATATTATAAATATAATTATCTTAAGGATTTTTTATTTTTTTTTTTTTATGTCCAAGT

4081 GCCCACGTGAATTTGCTGGTGAAAGTTAGCACTTGTGTGTGAACTCTACTTCCTCTTGTG

4141 TGTTTTACCAAGTATTTATACTCTGGTGCAACTAACTACTGTGTGAGGAATTGGTCCATG

4201 TGCAATAAATACCAATGAAGCACAATCAAGATTATGTACTGTGTGTCTGTAAAGGGTCAG

4261 TGATAATGAAAAAGACAGTTTGTTTTGTTCAAAATATAGACTGGATTTCCCATAGAGCTC

4321 TTTTAATAGACTTTCATGACTCAATAACATAGCAAAATGCCTCCAGACCTAAATAAGGTG

4381 TTTACCTACTGAGAGCTGCAG

[**0401**] 5.8.6 Mouse β_1-AR

[**0402**] The GenBank™ Accession No. for the Mus musculus β_1-AR gene is L10084. The Open Reading Frame extends from nucleotide 100 to nucleotide 1500 (shown in bold) (SEQ ID NO: 193)

1 GACCTCCCCGCGCGGGCCCCGCAGCCCCGGCTCCTGGGGTGCTCCCCAGGCGCGGCCCAG

61 CCCCGCCACACCCCCCGCCCCCGGCCTCCGCAGCTCGGC**ATGGGCGCGGGGGCGCTCGCC**

121 **CTGGGCGCCTCCGAACCCTGCAACCTGTCGTCCGCCGCGCCGCTGCCCGACGGTCCGGCC**

181 **ACCGCGGCGCGGCTGCTGGTGCTCGCGTCGCCTCCCGCCTCGCTGCTGCCTCCAGCCAGC**

241 **GAGGGCTCAGCGCCGCTGTCGCAGCAGTGGACCGCGGGTATGGGCCTACTCGTGGCGCTC**

301 **ATCGTTCTGCTCATCGTGGTGGGTAACGTGCTGGTGATCGTGGCCATCGCCAAGACCCCG**

361 **CGGCTGCAGACGCTCACCAACCTCTTCATCATGTCCCTGGCCAGCGCTGATCTGGTCATG**

421 **GGATTGCTGGTGGTGCCTTTCGGGGCCACCATCGTGGTGTGGCGCCCCTGGGAGTACGGC**

481 **TCCTTCTTCTGCGAGCTCTGGACTTCGGTAGATGTGCTGTGTGTGACGGCCAGCATTGAG**

541 **ACCCTGTGTGTCATCGCCCTGGACCGCTACCTCGCCATCACGTCGCCCTTTCGCTACCAG**

601 **AGTTTGCTGACGCGCGCGCGAGCGCGGGCCCTCGTGTGCACAGTGTGGGCCATCTCGGCG**

661 **TTGGTGTCCTTCCTGCCCATCCTCATGCACTGGTGGCGGGCCGAGAGCGACGAACCGCGC**

721 **CGCTGCTACAACGACCCCAAGTGCTGCGATTTCGTCACCAACAGGGCCTACGCCATCGCC**

781 **TCGTCCGTCGTCTCCTTCTACGTGCCCCTGTGCATCATGGCCTTCGTGTACCTGCGGGTG**

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 841 TTCCGCGAGGCCCAAAAACAGGTAAAGAAGATCGACAGCTGCGAGCOCCGCTTCCTCGGC

 901 GGCCCAGCCCGGCCGCCCTCGCCTGAGCCCTCGCCGTCACCTGGGCCACCGCGCCCCCGCA

 961 GACTCGCTGGCCAACGGGCGCTCCAGCAAGCGGCGGCCGTCGCGCCTCGTGGCTCTGCGC

1021 GAGCAGAAQGCGCTCAAGACACTGGGCATCATCATGGGTGTGTTCACGCTCTGCTGGCTG

1081 CCCTTCTTCCTGGCCAACGTGGTGAAGGCTTTCCACCGCGACCTGGTGCCCGATCGCCTC

1141 TTCGTCTTCTTCAACTGGCTGGGCTACGCCAACTCGGCCTTCAACCCCATCATCTACTGC

1201 CGCAGCCCCGACTTCCGCAAGGCTTTCCAGCGCCTGCTCTGCTGCGCGCGCCGGGCCCCC

1261 TGCAGACGCCGCGCAGCCCACGGGGACCGGCCGCGCGCCTCCCGCTGCCTGGCGAGAGCT

1321 GGGCCGCCGCCGTCCCCCGGAGCTCCCTCGGACGACGACGACGACGCCGGGACCACC

1381 CCACCGGCGCGCCTGCTGGAGCCCTGGACCCGCTGCAACGGCGGGACAACCACTGTGGAC

1441 AGCGATTCGAGCCTGGACGAGCCGGGGCGCCAGGGCTTCTCCTCGGAGTCCAAGGTGTAG

1501 AGAGCCAGGCTCTCTGGGCGCACGG
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[0403] 5.8.7 Frog β_1-AR

[0404] The GenBank™ Accession No. for the *Xenopus laevis* β_1-AR Gene is Y09213. The Open Reading Frame extends from nucleotide 301 to nucleotide 1458 (shown in bold) (SEQ ID NO:194)

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   1 ACCACCAACGTGGCACCTGCAGCTGAGGGACTAGAAAACTCCATAGCCCAGAGGAGTCAC

  61 TGGCAGCCACAACTGTACTGAAAGTGTAGCAGCTCACAAGCCCCCGGCTTATTCATCCAG

 121 GAGACAGAGAGACTGGCACAGTCCAGCCCAGTGCCACGAGAGTCTGCACCAACCAGGGGG

 181 AGTTATAGTTTCTGGACACAAGAGACTACCTGGCATCCCGCTGGCACCGACACTTTCTTT

 241 CTGTTCTGATATTCTGTTGCCCAAATGATCTGAGGCTCCAGGCTAGGACCTATGCCCATC

 301 ATGGGAGACGGTTGGGGGCCTATGGAGTGCAGGAACAGGTCTGGTACCCCTACAACAGTG

 361 CCCAGCCCTATGCACCCCCTGCCCGAGCTCACTCACCAGTGGACTATGGGAATGACTATG

 421 TTCATGGCGGCCATCATCCTCCTCATCGTCATGGGCAACATCATGGTCATTGTGGCCATT

 481 GGGAGGAACCAGAGGCTCCAGACCTTGACCAACGTCTTCATCACGTCCTTGGCTTGTGCC

 541 GACCTCATTATGGGTTTGTTTGTTGTGCCCCTTGGTGCCACGTTGGTGGTGAGTGGCAGG

 601 TGGCTGTACGGGTCGATATTCTGTGAGTTCTGGACGTCAGTGGACGTATTGTGCGTCACG

 661 GCGAGTATAGAGACCCTGTGCGTCATCTCCATCGACCGCTACATCGCCATCACCTCACCC

 721 TTCCGCTACCAGAGTCTCCTGACCAAGGGCCGTGCCAAGGGAATCGTGTGCAGCGTGTGG

 781 CGCATCTCAGCCCTGGTCTCGTTCCTGCCCATCATGATGCACTGGTGGAGGCACACTGGC

 841 GACCCCCTGGCCATGAAATGTTACGAGGATCCTGGGTGCTGTCATTTTGTCACCAACAGA

 901 GCTTACGCCATCGCCTCGTCCATCATCTCCTTCTATTTCCCACTCATCATCATGATCTTC

 961 GTCTACATCAGGGTCTTCAAGGAGGCGCAGAAGCAGATGAAGAAGATTGACAAGTGCGAG

1021 GGCAGGTTCTCCCATAGCCACGTCCTGAGCCACGGCAGGTCCAGCCGGAGGATCCTCTCC

1081 AAAATCCTGGTGCCCAAAGAGCAGAAAGCCTTGAAGACCCTCGGGATTATCATGGGCACC

1141 TTCACCCTGTGCTGGTTGCCCTTCTTCTTGGCCAACGTGGTCAATGTCTTCTACAGGAAC

1201 CTGATCCCAGACAAACTCTTCCTCTTCCTCAACTGGCTGGGCTACGCCAACTCCGCGTTT
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-continued

1261 **AACCCCATCATCTACTGCAGGAGCCCAGACTTCAGGAAGGCTTTCAAGAGACTCCTGTGT**

1321 **TGCCCCAAAAAGGCAGATCGGCACCTCCACACTACTGCGGAGCTCTCCCGATACTCGGGG**

1381 **GGCTTTGTTAACTCTTTAGACACCAATGCTTTGGGTATGTGTTCTGAATGTAATGGGGTG**

1441 **CGGACGTCATTGGACTGA**AATTAATTATTTATTGTGGGTCGGAGGGAGATTGAATAAGTG

1501 GGTGCGGGGCCCCAAATAACAGGTAGGTTCCAGGCAACCTCACTGCAGATTCTTGGAATG

1561 TAGAGGGTTCCCCAGGATAGGAGT

6.0 REFERENCES

[**0405**] The references listed below and all references cited herein are each specifically incorporated herein by reference in its entirety to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

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[0613] All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims. Accordingly, the exclusive rights sought to be patented are as described in the claims below.

What is claimed is:

1. An isolated antisense oligonucleotide at least 9 to about 35 nucleotides in length, wherein said oligonucleotide specifically binds to a portion of an mRNA expressed from a gene encoding a mammalian β_1-adrenoceptor polypeptide, and further wherein binding of said oligonucleotide to said mRNA is effective in altering the translation of said mRNA into said β_1-adrenoceptor polypeptide in a host cell expressing said mRNA.

2. The oligonucleotide of claim 1, comprising deoxyribonucleic acid, ribonucleic acid, or peptide-nucleic acid.

3. The oligonucleotide of claim 2, comprising a derivatized deoxyribonucleic acid, ribonucleic acid, or peptide-nucleic acid.

4. The oligonucleotide of claim 3, comprising a phosphorothioated deoxyribonucleic acid, ribonucleic acid, or peptide-nucleic acid derivative.

5. The oligonucleotide of claim 1, comprising a sequence of at least nine contiguous bases from any one of SEQ ID NO:4 through SEQ ID NO: 186.

6. The oligonucleotide of claim 5, comprising a sequence of at least ten contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID, NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or any one of SEQ ID NO:93 through SEQ ID NO:186.

7. The oligonucleotide of claim 6, comprising a sequence of at least thirteen contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or any one of SEQ ID NO:93 through SEQ ID NO: 186.

8. The oligonucleotide of claim 7, comprising a sequence of at least sixteen contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or any one of SEQ ID NO: 111 through SEQ ID NO: 186.

9. The oligonucleotide of claim 8, comprising a sequence of at least eighteen contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or any one of SEQ ID NO:127 through SEQ ID NO:186.

10. The oligonucleotide of claim 9, comprising a sequence of at least twenty contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:1, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or any one of SEQ ID NO:147 through SEQ ID NO:186.

11. The oligonucleotide of claim 10, comprising a sequence of at least twenty-two contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, or any one of SEQ ID NO:166 through SEQ ID NO:186.

12. The oligonucleotide of claim 11, comprising a sequence of at least twenty-four contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:181, SEQ ID NO:182, SEQ ID NO:183, SEQ ID NO:184, SEQ ID NO:185 or SEQ ID NO:186.

13. The oligonucleotide of claim 12, comprising a sequence of at least twenty-seven contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, or SEQ ID NO:28.

14. The oligonucleotide of claim 13, comprising a sequence of at least thirty contiguous bases from any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:ll, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, or SEQ ID NO:28.

15. The oligonucleotide of claim 14, comprising the sequence of any one of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, or SEQ ID NO:13.

16. The oligonucleotide of claim 14, comprising the sequence of any one of SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, or SEQ ID NO:23.

17. The oligonucleotide of claim 14, comprising the sequence of any one of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, or SEQ ID NO:28.

18. The oligonucleotide of claim 1, wherein said oligonucleotide is at least 90% complementary to the portion of the mRNA sequence to which it specifically binds.

19. The oligonucleotide of claim 18, wherein said oligonucleotide is at least 95% complementary to the portion of the mRNA sequence to which it specifically binds.

20. The oligonucleotide of claim 19, wherein said oligonucleotide is at least 98% complementary to the portion of the mRNA sequence to which it specifically binds.

21. The oligonucleotide of claim 1, wherein said oligonucleotide has no more than one nucleotide for every nine contiguous nucleotides that is non-complementary to the portion of the mRNA sequence to which it specifically binds.

22. The oligonucleotide of claim 21, wherein said oligonucleotide has no more than one nucleotide for every ten contiguous nucleotides that is non-complementary to the portion of the mRNA sequence to which it specifically binds.

23. The oligonucleotide of claim 22, wherein said oligonucleotide has no more than one nucleotide for every eleven contiguous nucleotides that is non-complementary to the portion of the mRNA sequence to which it specifically binds.

24. The oligonucleotide of claim 23, wherein said oligonucleotide has no more than one nucleotide for every twelve

contiguous nucleotides that is non-complementary to the portion of the mRNA sequence to which it specifically binds.

25. The oligonucleotide of claim 1, wherein said host cell is a mammalian host cell.

26. The oligonucleotide of claim 25, wherein said host cell is a human cell.

27. The oligonucleotide of claim 1, wherein said host cell is comprised within a human.

28. The oligonucleotide of claim 1, wherein said oligonucleotide is at least 9 to about 30 bases in length.

29. The oligonucleotide of claim 28, wherein said oligonucleotide is at least 9 to about 25 bases in length.

30. The oligonucleotide of claim 29, wherein said oligonucleotide is at least 9 to about 20 bases in length.

31. The oligonucleotide of claim 30, wherein said oligonucleotide is at least 9 to about 15 bases in length.

32. The oligonucleotide of claim 31, wherein said oligonucleotide is at least 9 to about 12 bases in length.

33. The oligonucleotide of claim 1, wherein said oligonucleotide comprises the sequence of any one of SEQ ID NO:29 to SEQ ID NO: 186.

34. The oligonucleotide of claim 33, wherein said oligonucleotide comprises the sequence of any one of SEQ ID NO:93 to SEQ ID NO: 186.

35. The oligonucleotide of claim 34, wherein said oligonucleotide comprises the sequence of any one of SEQ ID NO:93 to SEQ ID NO: 166.

36. The oligonucleotide of claim 35, wherein said oligonucleotide comprises the sequence of any one of SEQ ID NO:93 to SEQ ID NO:146.

37. The oligonucleotide of claim 36, wherein said oligonucleotide comprises the sequence of any one of SEQ ID NO:93 to SEQ ID NO: 126.

38. The oligonucleotide of claim 36, wherein said oligonucleotide comprises the sequence of any one of SEQ ID NO:96 to SEQ ID NO: 112.

39. A recombinant vector comprising (a) the oligonucleotide of claim 1, or (b) a polynucleotide that comprises a sequence that is complementary to the sequence of from about position 87 to position 1520 of SEQ ID NO: 187.

40. The vector comprising a viral, retroviral, adenoviral, or adenoassociated viral vector.

41. A host cell comprising the oligonucleotide of claim 1 or the recombinant vector of claim 39.

42. A composition comprising the oligonucleotide of claim 1.

43. A kit comprising the oligonucleotide of claim 1 or the composition of claim 42.

44. The composition of claim 42, further comprising a pharmaceutical excipient.

45. The composition of claim 44, further comprising a liposome, a lipid particle, a lipid vesicle, a nanoparticle, a microparticle, a nanocapsule, a nanosphere, or a sphingosome.

46. The composition of claim 42, further comprising at least a second oligonucleotide of at least 9 to about 35 nucleotides in length, wherein said second oligonucleotide also specifically binds to a portion of an mRNA that encodes a β_1-adrenoceptor polypeptide, and further wherein the nucleotide sequence of said second oligonucleotide is different from the nucleotide sequence of the first oligonucleotide in said composition.

47. The composition of claim 46, wherein said second oligonucleotide is any one of SEQ ID NO:4 through SEQ ID NO:186.

48. The composition of claim 42, further comprising at least a first anti-hypertensive agent.

49. The composition of claim 48, wherein said anti-hypertensive agent is selected from the group consisting of captopril, enalapril, ramipril, cilazapril, fosinopril, and lisinopril.

50. The composition of claim 42, further comprising at least a second antisense oligonucleotide specific for a mammalian transcription factor mRNA, a reninspecific mRNA, an angiotensin-specific mRNA, an angiotensinogen-specific mRNA, an AT-1 receptor-specific mRNA, or an angiotensin converting enzyme.

51. The composition of claim 50, wherein said second antisense oligonucleotide is specific for a mammalian angiotensin converting enzyme mRNA.

52. A method for reducing the level of β_1-adrenoceptor polypeptide in a mammalian host cell, said method comprising providing to said cell, an amount of the oligonucleotide of claim 1, or the composition of claim 42, and for a time effective to reduce the level of said polypeptide in said host cell.

53. A method for reducing the level of transcription of a β_1-adrenoceptor polypeptide-specific mRNA in a mammalian host cell, said method comprising providing to said cell, an amount of the oligonucleotide of claim 1, or the composition of claim 42, and for a time effective to reduce the level of transcription of said mRNA in said host cell.

54. The method of claim 52 or **53**, wherein said host cell is comprised within a mammal.

55. The method of claim 54, wherein said mammal is a human.

56. A method for decreasing hypertension in a mammal, said method comprising administering to said mammal, an amount of the oligonucleotide of claim 1 or the composition of claim 42, and for a time effective to decrease hypertension in said mammal.

57. A method for reducing the symptoms of a disease or disorder associated with an elevated β_1-adrenoceptor polypeptide level in a mammal, said method comprising administering to said mammal a therapeutically effective amount of the oligonucleotide of claim 1, SEQ ID NO:1, SEQ ID NO:2, or SEQ ID NO:3, or the composition of claim 42, for a time sufficient to decrease the level of said polypeptide in said mammal, thereby reducing the symptoms of said disease or disorder in said mammal.

58. A method for treating myocardial ischemia or cardiac hypertrophy in an affected mammal, said method comprising administering to said mammal, a therapeutically effective amount of the oligonucleotide of claim 1, SEQ ID NO: 1, SEQ ID NO:2, or SEQ ID NO:3, or the composition of claim 42, for a time sufficient to treat said ischemia or said hypertrophy in said mammal.

59. A method for reducing the level of β_1-adrenoceptor polypeptide in a mammalian host cell, said method comprising expressing in said host cell, a selected genetic construct that comprises a full-length, or substantially full-length β_1-adrenoceptor antisense polynucleotide operably linked to a promoter capable of expressing said polynucleotide in said cell.

* * * * *