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Molecular and pharmacological characteristics of the gerbil α_{1A} -adrenergic receptor

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Abstract

The spiral modiolar artery supplies blood and essential nutrients to the cochlea. Our previous functional study indicates the α_{1A} -adrenergic receptor subtype mediates vasoconstriction of the gerbil spiral modiolar artery. Although the gerbil cochlea is often used as a model in hearing research, the molecular and pharmacological characteristics of the cloned gerbil α_{1A} -adrenergic receptor have not been determined. Thus we cloned, expressed and characterized the gerbil α_{1A} -adrenergic receptor and then compared its molecular and pharmacological properties to those of other mammalian α_{1A} -adrenergic receptors. The cDNA clone contained 1404 nucleotides, which encoded a 467 amino acid peptide with a deduced sequence having 96.8, 96.4 and 91.6% identity to rat, mouse and human α_{1A} -receptors, respectively. We transiently transfected the α_{1A} -adrenergic receptor into COS-1 cells and determined its pharmacological characteristics by [³H]prazosin binding. Unlabeled prazosin had a K_i of 0.89 ± 0.1 nM. The α_{1A} -adrenergic receptor-selective antagonists, 5-methylurapidil and WB-4101, bound with high affinity and had K_i values of 4.9 ± 1 and 1.0 ± 0.1 nM, respectively. BMY-7378, an α_{1D} -adrenergic receptor-selective antagonist, bound with low affinity (260 ± 60 nM). The 91.6% amino acid sequence identity and K_i s of the cloned gerbil α_{1A} -adrenergic receptor are similar to those of the human α_{1A} -adrenergic receptor clone. These results show that the gerbil α_{1A} -adrenergic receptor is representative of the human α_{1A} -adrenergic receptor, lending validity to the use of the gerbil spiral modiolar artery as a model in studies of vascular disorders of the cochlea.

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Keywords

Alpha₁-adrenergic receptor subtype; Gerbil cochlea; Spiral modiolar artery

1. Introduction

The cochlea is the hearing component of the inner ear. It translates mechanical energy from movement of middle ear bones into nerve impulses, which are ultimately transmitted to the brain where sound is perceived. The cochlea receives its blood supply from the spiral modiolar artery and the cochlear branch of the vestibulocochlear artery. Cochlear viability depends on adequate blood flow through these arteries (Seidman et al., 1999). For example, experimental interruption of blood flow to the inner ear results in ischemia-induced damage to the cochlea (Billett et al., 1989; Iwasaki et al., 1997; Koga et al., 2003), and disturbances in cochlear blood flow are implicated in sudden sensorineural hearing loss, presbycusis and noise-induced hearing loss in humans (Nakashima et al., 2003; Seidman et al., 1999; Shikowitz, 1991).

α_1 -Adrenergic receptors mediate constriction of various blood vessels and are therapeutic targets in the treatment of several cardiovascular diseases. By convention, native α_1 -adrenergic receptor subtypes are denoted by uppercase letters, while cloned α_1 -adrenergic receptor subtypes are indicated with lowercase letters (Hieble et al., 1995). α_1 -Adrenergic receptors are subdivided into α_{1A} -, α_{1B} - and α_{1D} -adrenergic receptor subtypes based on their molecular structure and pharmacological characteristics. Hence, subtype-selective antagonists, which have varying affinities for each subtype, can be used to distinguish the α_{1A} - from the α_{1B} - from the α_{1D} -adrenergic receptor. For example, at α_{1A} -receptors, 5-methylurapidil has a higher affinity than BMY-7378; while at α_{1B} -receptors, 5-methylurapidil and BMY-7378 have similar affinities; and at α_{1D} -receptors, BMY-7378 has a higher affinity than 5-methylurapidil (Bockman et al., 2004; Goetz et al., 1995; Gross et al., 1988).

Because cochlear function is particularly sensitive to ischemic conditions, it is important to understand the mechanisms that regulate vascular tone of arteries supplying the cochlea. The spiral modiolar artery is innervated by norepinephrine-containing sympathetic nerves (Carlisle et al., 1990), and vasoconstriction of the spiral modiolar artery is mediated by α_1 -adrenergic receptors (Gruber, 1998). In addition, α_1 -adrenergic receptors have been shown to regulate cochlear blood flow (Ohlsen et al., 1991). Thus, α_1 -adrenergic receptors on the spiral modiolar artery may be important in the pathophysiology of sensorineural hearing loss caused by impaired cochlear blood flow.

Consistent blood flow measurements are possible in the gerbil because of its large ear parts and thin cochlear bone (Mom et al., 1999), and the vascular anatomy of gerbil and human cochleae are similar. Consequently, the gerbil is widely used as a model of cochlear blood flow (Nakashima et al., 2003). It is therefore important to understand the regulatory mechanisms controlling gerbil cochlear vasculature. Previously, we reported that reverse transcription-polymerase chain reaction identified mRNA for only the α_{1A} -adrenergic receptor subtype in gerbil spiral modiolar arteries (SMA). In addition, functional

characterization of the α_1 -subtype on the SMA showed that 5-methylurapidil blocked norepinephrine-induced contraction with relative high affinity, indicating the α_{1A} -adrenergic receptor subtype regulates contraction of the SMA (Gruber et al., 1998).

It is important to know whether or not the gerbil α_{1A} -adrenergic receptor subtype is typical of α_{1A} -subtypes from other species, particularly human, because of its role in a key model of cochlear blood flow. Thus, we first cloned the gerbil α_{1A} -adrenergic receptor to determine its molecular properties, and then compared its deduced amino acid sequence to those of cloned α_{1A} -adrenergic receptors from other species. Secondly, we used radioligand binding to directly determine the pharmacological characteristics of the expressed gerbil α_{1A} -receptor, and then compared the affinities (K_i values) to those of other mammalian $\alpha_{1A/A}$ -adrenergic receptors.

2. Materials and Methods

2.1 Tissue isolation and total RNA extraction

Animal experiments were carried out in accordance with the European Commission Directive 86/609. Gerbils were anesthetized with pentobarbital (50 mg/kg, i.p.) and then decapitated with approval by Creighton University's Institutional Animal Care and Use Committee. Spiral modiolar arteries (SMA) were removed, frozen in liquid nitrogen and stored at -70°C . Total RNA was extracted with TRIzol Reagent (Invitrogen).

2.2 Amplification of gerbil α_{1A} -adrenergic receptor cDNA by RT-PCR

Approximately 1 μg of SMA total RNA was reverse transcribed in 20 μl containing 20 mM Tris HCl (pH 8.4), 50 mM KCl, 2.5 mM MgCl_2 , 25 pmol antisense gene-specific primer, 20 units RNasin, 1 mM dNTP and 50 units murine leukemia virus reverse transcriptase and incubated for 50 min at 42°C , followed by 5 min at 99°C in a PerkinElmer 480 DNA Thermal Cycler. The polymerase chain reaction (PCR) was performed on 10 μl of the reverse-transcribed cDNA reaction containing 25 pmol of sense primer and 2.5 units of Platinum *Pfx* DNA polymerase (Invitrogen). PCR conditions included denaturation for 5 min at 95°C , followed by 39 one-minute cycles at 95°C , 1 min at 58°C , 3 min at 72°C and a final extension of 10 min at 72°C . RT-PCR products were visualized on a 3% agarose gel stained with ethidium bromide and documented with the 1000 gel documentation system (Bio-Rad, Hercules, CA).

Table 1 lists oligonucleotide sense and antisense primers used in RT-PCR. Primer designs were based on those previously described (Scofield et al., 1995) and the third cytoplasmic loop of the gerbil α_{1A} -adrenergic receptor (Gruber et al., 1998). In addition, a degenerative primer was used to amplify the 5' region of the gerbil α_{1A} -adrenergic receptor, and a 3' RACE (FirstChoice RLM-RACE Kit, Ambion, Austin, TX) with gerbil-specific primers from regions already sequenced was used to obtain the C-terminal sequence. PCR and 3' RACE products were then cloned into the pCRII cloning vector using a TA Cloning Kit (Invitrogen). Clones were sequenced (ABI model 373, Life Technologies Corporation, Carlsbad, CA) and analyzed using the Wisconsin Package Version 10.1 software (Genetics Computer Group, Madison, WI).

2.3 Cloning of full length gerbil α_{1a} -adrenergic receptor cDNA into an expression vector

The full length gerbil α_{1a} -adrenergic receptor from total RNA was amplified by RT-PCR with primers UP4 and DN3 (Figure 1, Table 1) as described above, except Platinum *Pfx* DNA polymerase (Invitrogen) with 2.5 mM $MgSO_4$ was used. Restriction digest sites and a Kozak sequence were then added by performing PCR with the sense primer UP-EcoRI containing two *EcoR* I digest sites and a Kozak sequence GCCACC, and the antisense primer DN-NotI containing two *Not* I digest sites (Table 1) (Kozak, 1987). The PCR product was cloned into the pcDNA3.1+ vector (Invitrogen) using restriction digest sites *EcoR* I and *Not* I. The DNA was sequenced and analyzed.

2.4 Transfection of recombinant gerbil α_{1a} -adrenergic receptor

COS-1 cells in Dulbecco's Modified Eagle's medium supplemented with 10% fetal bovine serum were grown to confluence in T-75 cell culture flasks at 37°C in a humidified 95% air, 5% CO₂ incubator. Recombinant gerbil α_{1a} -adrenergic receptor was transiently transfected into several flasks of COS-1 cells using FuGENE 6 Reagent with a 2:1 ratio according to the manufacturer's protocol (Roche, Indianapolis, IN). To confirm transfection of the recombinant gerbil α_{1a} -adrenergic receptor, receptor expression (fmol/mg protein) was quantified by [³H]prazosin binding (See Subsection 2.5). We determined specific binding (total minus nonspecific binding in the presence of 10 μ M phentolamine) of a single concentration of [³H]prazosin and then calculated receptor expression according to the law of mass action. α_{1a} -Adrenergic receptor expression in the transfected COS-1 cells was 560 $\pm$ 95 fmol/mg protein, n = 3.

2.5 Cell membrane preparation and radioligand binding

Cell membrane preparation and radioligand binding were performed as described previously (Bockman et al., 2004). Briefly, 48 hr after transfection, cells were rinsed with phosphate-buffered saline, scraped and then harvested by centrifugation at 4°C for 15 minutes at 1000g. The pellet was suspended in ice-cold 50 mM Tris buffer (pH 7.4), and then homogenized twice at 22,000 rpm for 10 seconds. The homogenate was centrifuged at 4°C for 20 minutes at 30,000g. After resuspending the membrane pellet in Tris buffer, it was washed twice by centrifugation and stored at -70°C. The pellet was resuspended in 50 mM Tris buffer and homogenized prior to analysis. Protein concentrations were determined by the Bradford assay (Pierce, Rockford, USA) (Bradford, 1976).

Membranes expressing transfected α_{1a} -adrenergic receptor were incubated with [³H]prazosin in the presence of increasing concentrations of unlabeled drugs for 30 min at 37°C. Membrane suspensions were separated by vacuum filtration through GF/B glass microfiber filters and then rinsed three times with 5 ml of ice-cold 50 mM Tris buffer. Radioactivity retained on the filters was counted by liquid scintillation spectroscopy. Radioligand binding data were analyzed by nonlinear least-squares curve fitting to determine IC₅₀ values. K_i values were calculated from IC₅₀ values (Cheng and Prusoff, 1973).

2.6 Spiral Modiolar Artery Contraction and Chloroethylclonidine Inactivation of α_{1A} -Adrenergic receptors

Concentration-response curves for norepinephrine in causing contraction of spiral modiolar arteries were obtained as previously described (Gruber et al., 1998). In the current report, the diameters of artery segments were between 55 and 67 μm at rest. Norepinephrine caused a mean maximal reduction in the vascular diameter of $12 \pm 2\%$.

Chloroethylclonidine (CEC) irreversibly inactivates α_{1A} -adrenergic receptors, which results in a reduction in their receptor number. α_{1A} -Adrenergic receptors mediating contraction of the spiral modiolar artery were inactivated by pretreatment with CEC as formerly described (Xiao et al., 1998). For the present study, control concentration-response curves for norepinephrine-induced contraction were generated and subsequently washed out for 30 min. Arteries were then incubated with 100 μM CEC for 2, 5, 7 or 10 min, followed by 45 min of extensive washing to remove any unbound CEC. Concentration-response curves to norepinephrine were then repeated. Concentration-response curves were analyzed using non-linear least squares curve fitting to determine the concentration of norepinephrine causing 50% of the maximal response (EC_{50}). Maximal contraction and EC_{50} values before and after CEC treatment were compared using a Student's *t*-test with a value of $P < 0.05$ accepted as a significant difference between groups. Values for maximal contraction and EC_{50} are means $\pm$ S.E.

2.7 Materials

BMY-7378 dihydrochloride, chloroethylclonidine dihydrochloride, 5-methylurapidil, (–)-norepinephrine bitartrate, phentolamine methanesulfonate, prazosin hydrochloride, and WB-4101 hydrochloride were obtained from Sigma-Aldrich (St. Louis, MO). [7-methoxy- ^3H]prazosin ([^3H]prazosin) (70–87 Ci/mmol) was obtained from PerkinElmer (Boston, MA). *EcoR* I and *Not* I were purchased from Invitrogen (Carlsbad, CA) and New England Biologicals (Ipswich, MA), respectively.

3. Results and Discussion

3.1 Molecular characteristics of the gerbil α_{1a} -adrenergic receptor clone

In the present study, the gerbil α_{1a} -adrenergic receptor was cloned from total RNA by RTPCR using gene-specific primers. Previously, we amplified a 175-nucleotide sequence from the third cytoplasmic loop of the gerbil α_{1a} -adrenergic receptor in spiral modiolar arteries (Gruber et al., 1998). In addition to these primers directed against the third cytoplasmic loop, we used formerly described primers (Scofield et al., 1995) based on α_{1a} -adrenergic receptor sequences from other species to clone portions of the gerbil α_{1a} receptor (Table 1, Figure 1). Consequently, the 5' region was cloned with a degenerate primer (UP2) from the open reading frame start site based on consensus sequences for the α_{1a} -adrenergic receptor from rat, mouse, human and cow. The RT-PCR product generated from the UP2 and DN2 primers is shown in Figure 2.

The 3' region of the gerbil α_{1a} -adrenergic receptor was amplified by a 3' RACE using the gene-specific primer UP3. The complete cDNA sequence was amplified by RT-PCR with

primers UP4 and DN3, and the resulting PCR product (Figure 2) was cloned and sequenced (Table 2). The cDNA clone of the complete gerbil α_{1A} -receptor consisted of 1404 nucleotides with a 1401 bp open reading frame, which had a 93.0, 93.1 and 88.1% identity compared to the cDNA nucleotide sequences of rat, mouse and human α_{1A} -adrenergic receptors, respectively. The coding region encoded a 467 amino acid peptide with an overall amino acid composition exhibiting a high degree of sequence homology with those of α_{1A} -adrenergic receptors from three other species. The deduced amino acid sequence of the full-length gerbil α_{1A} receptor was 96.8, 96.4 and 91.6% identical to the amino acid sequences of rat, mouse and human α_{1A} -receptors, respectively (Table 3).

α_1 -Adrenergic receptor subtypes are members of the G protein-coupled family of signaling proteins and mediate the actions of the sympathetic neurotransmitter norepinephrine. α_1 -Adrenergic receptors consist of seven transmembrane domains containing three cytoplasmic loops. The amino acid sequences of the seven transmembrane domains are highly conserved among species (Zhong and Minneman, 1999). In our study (Table 3), alignment of the amino acid residues of the gerbil α_{1A} -receptor with those of cloned α_{1A} -receptors from various species revealed greater than 95% identity of the seven membrane spanning domains between gerbil, rat, mouse and human. These results are consistent with the structural homology of the transmembrane domains of the α_{1A} -receptor subtype from various species. In contrast, sequence differences commonly occur in the third cytoplasmic loop (Zhong and Minneman, 1999). In the present report, alignment of the amino acid sequences between species showed an additional glycine residue in the third cytoplasmic loop of the gerbil α_{1A} -receptor (Table 3).

3.2 Pharmacological characteristics of the gerbil α_{1A} -adrenergic receptor clone

The recombinant gerbil α_{1A} -adrenergic receptor was transfected into COS-1 cells, and then directly examined in membranes by radioligand binding with [3 H]prazosin (Figure 3). The pharmacological characteristics of the gerbil α_{1A} -adrenergic receptor were determined and compared to those of other mammalian $\alpha_{1A/A}$ -adrenergic receptors (Table 4). For example, affinities (K_i values) of α_1 -adrenergic receptor antagonists were obtained from competition curves for inhibition of [3 H]prazosin binding by prazosin (nonselective), WB-4101 and 5-methylurapidil (α_{1A} -selective) and BMY-7378 (α_{1D} -selective) (Figure 3). K_i values in nM were 0.89 ± 0.09 for prazosin, 1.0 ± 0.1 for WB-4101, 4.9 ± 1 for 5-methylurapidil and 260 ± 60 for BMY-7378. The rank order of affinity values: prazosin > WB-4101 > 5-methylurapidil > BMY-7378 and the absolute affinity values are typical for the α_{1A} -adrenergic receptor subtype across species. Table 4 compares our K_i values for these antagonists in the gerbil with published K_i values in human, mouse and rat and indicates the similarity between the pharmacological characteristics of the gerbil and human α_{1A} -adrenergic receptor subtypes.

3.3 Effect of chloroethylclonidine on the α_{1A} -adrenergic receptor-mediated vascular response of spiral modiolar arteries

Figure 4 illustrates mean concentration-response curves for norepinephrine in causing contraction of gerbil spiral modiolar arteries before and after pretreatment with 100 μ M chloroethylclonidine (CEC) for 2, 5, 7 or 10 min. CEC pretreatment significantly inhibited

maximal contractile responses to norepinephrine in a time-dependent manner. Norepinephrine-induced maximal contraction was reduced by $34 \pm 13\%$ at 2 min, $62 \pm 10\%$ at 5 min, $67 \pm 5\%$ at 7 min and $80 \pm 4\%$ at 10 min compared to the control maximal response, $P < 0.05$. These results are consistent with the kinetics of inactivation by CEC of α_{1A} -adrenergic receptors (Xiao et al., 1998). Conversely, CEC pretreatment for 2, 5, 7 or 10 min had no effect on EC_{50} values of norepinephrine-induced contraction compared with the control EC_{50} , $P > 0.05$ (See Figure 4 legend for mean EC_{50} values).

Chloroethylclonidine (CEC) irreversibly inactivates α_{1A} -adrenergic receptors, which results in a reduction in their receptor number (Bockman et al., 2004; Han et al., 1987; Xiao et al., 1998). We used CEC to inactivate α_{1A} -adrenergic receptors and thus determine whether or not there is a receptor reserve for norepinephrine in causing α_{1A} -adrenergic receptor-mediated contraction of the gerbil spiral modiolar artery. A receptor reserve is present when an agonist elicits a maximal response even after receptor inactivation results in reduction in receptor number (Furchgott, 1966; Ruffulo, 1982). In the present study, receptor inactivation with CEC resulted in inhibition of the maximal response to norepinephrine without a concomitant rightward shift of the concentration-response curve. Consistent with classical receptor theory, these data indicate that there is no receptor reserve for norepinephrine-induced contraction of the spiral modiolar artery (Ruffulo, 1982).

A receptor reserve is an important physiological mechanism regulating the response to neurotransmitters and other agonists (Ruffulo, 1982). For example, the presence of a receptor reserve provides a mechanism for a tissue to maintain its capacity to respond when disease or chronic drug treatment downregulates receptor number. In the present study, there was no α_{1A} -adrenergic receptor reserve for norepinephrine-induced contraction. Thus, the contractile response of the spiral modiolar artery would be reduced by loss of receptor number, which may have important implications for cochlear blood flow. Although we did not examine cochlear blood flow, the demonstration of a lack of receptor reserve in an artery that is thought to contribute to cochlear blood flow adds to our understanding of the mechanisms controlling cochlear vasculature.

3.4 Conclusions

In addition to the rat, mouse and guinea pig, the gerbil is often used as a model in studies of the cochlear vasculature. The α_{1A} -adrenergic receptor mediates vasoconstriction of the gerbil spiral modiolar artery, and the spiral modiolar artery regulates cochlear blood flow. However, the α_1 -adrenergic receptor subtype in the spiral modiolar artery has not been directly studied. Thus, we have cloned the recombinant gerbil α_{1a} -adrenergic receptor and characterized its molecular and pharmacological properties. In summary, the 91.6% amino acid sequence identity and K_i s of the cloned gerbil α_{1a} -adrenergic receptor are similar to those of the human α_{1a} -adrenergic receptor. These results show that the gerbil α_{1a} -adrenergic receptor is typical of the human α_{1a} -adrenergic receptor. Further, the results of the experiments with the inactivating agent, chloroethylclonidine, which show a lack of receptor reserve, indicate that norepinephrine-induced contraction of the spiral modiolar artery is tightly regulated by a limited number of α_{1A} -adrenergic receptors. Thus, the gerbil

spiral modiolar artery provides a useful model for further examining the role of α_{1A} – adrenergic receptors in sensorineural hearing loss caused by impaired cochlear blood flow.

Abbreviations

CEC	Chloroethylclonidine
5-MU	5-methylurapidil
RT-PCR	reverse transcription-polymerase chain reaction
RACE	rapid amplification of cDNA ends
SMA	spiral modiolar artery

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Research Highlights

- gerbil and human α_{1a} -adrenergic receptors share 91.6% amino acid sequence identity
- K_i s of gerbil and human α_{1a} -adrenergic receptor are similar
- gerbil α_{1a} -adrenergic receptor is representative of a human α_{1a} -adrenergic receptor
- gerbil spiral modiolar artery is a model of α_{1A} receptors and cochlear blood flow

Figure 1.

Oligonucleotide primers and the corresponding PCR products. Upstream sense primers are UP1, UP2, UP3 and UP4; and downstream antisense primers are DN1, DN2 and DN3. Primer locations with respect to their complementary cDNA sequence are indicated by boxes. Primer sequences and species specificity are listed in Table 1. PCR products, their size and respective locations on the gerbil α_{1a} -adrenergic receptor gene are indicated by arrows.

Figure 2.

RT-PCR products from gerbil total RNA were amplified with indicated sense (UP) and antisense (DN) primers from Table 1. Lane 1: 215 bp product of primers UP1+DN1. Lane 2: 1305 bp product of primers UP2+DN2. Lane 3: 100 bp DNA ladder. Lane 4: 219 bp product of primers UP3+DN3. Lane 5: 1453 bp product of primers UP4+DN3.

Figure 3.

Mean competition binding curves showing α_1 -adrenergic receptor antagonist inhibition of [7-methoxy- ^3H]prazosin (^3H -prazosin) binding in membranes from COS-1 cells transiently transfected with gerbil α_{1a} -adrenergic receptor. For each concentration of antagonists, [^3H]prazosin binding is expressed as a percentage of the specific binding in the absence of any drug.

Figure 4.

Mean concentration-response curves for norepinephrine in causing contraction of gerbil spiral modiolar arteries before (Control) and after pretreatment with 100 μ M chloroethylclonidine (CEC) for 2, 5, 7 or 10 minutes (Min). Contractile responses are normalized as the percent of maximal contraction to norepinephrine in the Control. Each curve represents mean responses of four to seven individual arteries each taken from different animals. CEC pretreatment significantly inhibited maximal contractile responses to norepinephrine compared to the control maximal response by $34 \pm 13\%$ (2 Min CEC), $62 \pm 10\%$ (5 Min CEC), $67 \pm 5\%$ (7 Min CEC) and $80 \pm 4\%$ (10 Min CEC), $P < 0.05$. Mean EC_{50} values for norepinephrine-induced contraction were $21 \pm 3 \mu$ M (Control), $30 \pm 6 \mu$ M (2 Min CEC), $29 \pm 9 \mu$ M (5 Min CEC), $42 \pm 10 \mu$ M (7 Min CEC) and $30 \pm 10 \mu$ M (10 Min CEC). Mean EC_{50} values after CEC pretreatment were not different from the mean control EC_{50} , $P > 0.05$.

Table 1

Sequence and species specificity of oligonucleotide primers. Y=C/T, K=T/G, and R=A/G.

Primer	Sequence	Species
UP1	5'-GTAGCCAAGAGAGAAAAGCCG-3'	Rat/ Mouse
DN1	5'-CAACCCACCACGATGCCCAG-3'	Rat
UP2	5'-ATGGTGYTTCTYTCKGAAA-3'	Rat/Mouse/Human/Cow
DN2	5'-GCAGCAGACCTGCAAAAAG-3'	Rat
UP3	5'-AGGGATCGGCCAGGATTACA-3'	Rat/ Gerbil
DN3	5'-ATCAGCAGGACCTAGCGTCAA-3'	Gerbil
UP4	5'-ATGGTGTTCCTTCGGAAAATGC-3'	Gerbil
UP-EcoRI	5'-GAATTCGAATTCGCCACCATGGTGTTCCTTCGGAAAA-3'	Gerbil
DN-NotI	5'-GCGGCCGCGCGGCCGCTAGACTTCCTCCCCGTTTCG-3'	Gerbil

Table 2

Nucleotide and amino acid sequences of the gerbil α_{1A} -adrenergic receptor (accession number HM754402).

1	ATGGTGTTCCTTTGCGAAATGCTTCGAGGGCTCCAACTGCACCCAGCCAGCAGCGGTGAACATTTCTAAGGCCATTCGCTTGGG	90
	M V F L S E N A S E G S N C T H Q P A P V N I S K A I L L G	
91	GTAATCTTTGGGGGCTCATCATCTTCGGGTCTCGGGAACTCTTACTGATCTTTGGGTGGCTGTCTATCGGCACCTGCATCAGTG	180
	V I L G G L I I F G V L G N I L V I L S V A C H R H L H S V	
181	ACTCACTACATAGTCAACCTGGCGCTCGCGACCTCTCTCTCACTCCACCTGCTGCGCTTCTCGGCATCTTTGAGATCTGGGC	270
	T H Y Y I V N L A V A D L L L T S T V L F F S A I F E I L G	
271	TACTGGGCTTTGGCAGGTCTCTGCAACATCTGGGCTGCGGTGGACCTCTGTGCTGCACGGGCTCCATATGGGCTCTGCATCATC	360
	Y W A F G R V F C N I W A A V D V L C C T A S I M G L C I I	
361	TCCATCGACATACATTTGGGTGAGCTACCGCTGCGCTACCTGCCATTGTCACCAGAGGAGGGGCTCAGGGCTCTGCTCTGCGTC	450
	S I D R Y I G V S Y P L R Y P A I V T Q R R G V R A L L C V	
451	TGGCGCTTTCCCTGGTCATCTCCATCGACCGTTGTTGGCTGGAGGAGCGGCTCCTGAGGATGAGCAATCTGCCAGATCAATGAG	540
	W A L S L V I S I G P L F G W R Q P A P E D E T I C Q I N E	
541	GAGCTGGCTTACGTCTGTTCTCCGGCTGGGCTCTTTCTACGTGCCCTGGCCATCATCTGGTTATGTACTGTCGGGTCTACGTGTA	630
	E P G Y V L F S A L G S F Y V P L A I I L V M Y C R V Y V V	
631	GCCAGAGGGAAAGCCGGGCTCAAGTCCGGCTCAAGACCGCAAGTCGGACTCAGAGCAAGTGAGCTCCGTATCCACCGGAAAAAT	720
	A K R E S R G L K S G L K T D K S D S E Q V T L R I H R K N	
721	GTCCCCGAGAAGCGCGGAGGCTGAGCAGTGCCAGAATAAGACTCACTTCTCCGTGGGCTGCTCAAGTTTCCGGGAGAAAAA	810
	V P A E G G G G L S S A K N K T H F S V R L L K F S R E K K	
810	GCGGCCAAGACGCTGGGCATCGTGGTGGTCTTTGTCTCTGCTGGCTGCCCTCTTCTAGTATGCCATTTGGGTCTCTTCCCG	900
	A A K T L G I V V G C F V L C W L P F F L V M P I G S F P P	
901	GATTTCAAGCTCCGGAAACGTTTCAAAATAGTGTTTGGCTCGGTACCTCACAGCTGCATCAACCCCATCATATACCATGCTCC	990
	D F K P P E T V F K I V F W L G Y L N S C I N P I I Y P C S	
991	AGCCAAAGTTCAGAAAGCTTTTCAGAATGCTCAGAAATCAGTGTCTCCGAGAAGCCGCTTCCAAAGCATGCCCTGGGCTACACC	1080
	S Q E F K K A F Q N V L R I Q C L R R R P S S K H A L G Y T	
1081	CTGCACCCGCCAGCCAGGCTGTAGAAGGACAGCAGAGGCATGGTGGTATCCCGGTGGGCTCGGAGAGACTTTCTATAAGATCTCC	1170
	L H P P S Q A V E G Q H R G M V R I P V G S G E T F Y K I S	
1171	AAAGCGAGCGAGCTCTGTGAATGGAAGCTTCTCTCTCCATGCCCGAGGGTCAAGCAGGATTACAGTGCCGAGGACCAATCTGCTGC	1260
	K A D G V C E W K L L S S M P Q G S A R I T V P K D Q S A C	
1261	ACCACCGCCCGGTGAGAATAAAGCTTTTTCAGGTCTGCTGCTGTGTGGGTCTCGACCCCGAGCTTGAAGAAAATCACAGGTT	1350
	T T A R V R S K S F L Q V C C C V G S S T P S L E E N H Q V	
1321	CCACCATTAAGATCCACCATCTCCCTTGGCGAAACGGGGAGGAAGTCTAG	1404
	P T I K I H T I S L G E N G E E V *	

Table 3

Comparison of amino acid sequences of gerbil (accession number HM754402), rat (U071126), mouse (AF031431), and human (U02569) α_{1a} -adrenergic receptors.

	1	60
Gerbil	MVFLSENASEGSNCTHPPAPVNISKAILLGVILGGLIIFGVLGNIILVLSVACHRHLSH	
Rat	MVLLSENASEGSNCTHPPAPVNISKAILLGVILGGLIIFGVLGNIILVLSVACHRHLSH	
Mouse	MVLLSENASEGSNCTHPPAPVNISKAILLGVILGGLIIFGVLGNIILVLSVACHRHLSH	
Human	MVFLSGNASDSNCTHPPAPVNISKAILLGVILGGLIIFGVLCNIILVLSVACHRHLSH	
	61	120
Gerbil	THYYIVNLAVADLLLTSTVLPPSAIFEILGYWAFGRVFCNIWAADVLCCTASIMGLCTI	
Rat	THYYIVNLAVADLLLTSTVLPPSAIFEILGYWAFGRVFCNIWAADVLCCTASIMGLCTI	
Mouse	THYYIVNLAVADLLLTSTVLPPSAIFEILGYWAFGRVFCNIWAADVLCCTASIMGLCTI	
Human	THYYIVNLAVADLLLTSTVLPPSAIFEILGYWAFGRVFCNIWAADVLCCTASIMGLCTI	
	121	180
Gerbil	SIDRYIGVSYPLRYPTIVTQRRGVALLCWAALSLVISIGPLFGWRQPAPEDEETICQINE	
Rat	SIDRYIGVSYPLRYPTIVTQRRGVALLCWAALSLVISIGPLFGWRQPAPEDEETICQINE	
Mouse	SIDRYIGVSYPLRYPTIVTQRRGVALLCWAALSLVISIGPLFGWRQPAPEDEETICQINE	
Human	SIDRYIGVSYPLRYPTIVTQRRGVALLCWAALSLVISIGPLFGWRQPAPEDEETICQINE	
	181	240
Gerbil	EPGYVLFSAIGSFYVPLAILLVMYCRVYVAKRESRGLKSGLTKDSDSEQVTLRIHRKN	
Rat	EPGYVLFSAIGSFYVPLAILLVMYCRVYVAKRESRGLKSGLTKDSDSEQVTLRIHRKN	
Mouse	EPGYVLFSAIGSFYVPLAILLVMYCRVYVAKRESRGLKSGLTKDSDSEQVTLRIHRKN	
Human	EPGYVLFSAIGSFYVPLAILLVMYCRVYVAKRESRGLKSGLTKDSDSEQVTLRIHRKN	
	241	300
Gerbil	VPAEGGGGLSSAKNKTTHFSVRLKFSREKKAATLGIVVGCFLCWLPPFLVMPIGSFPP	
Rat	VPAEGG-GVSSAKNKTTHFSVRLKFSREKKAATLGIVVGCFLCWLPPFLVMPIGSFPP	
Mouse	VPAEGS-GVSSAKNKTTHFSVRLKFSREKKAATLGIVVGCFLCWLPPFLVMPIGSFPP	
Human	APAEGS-GMASAKTTHFSVRLKFSREKKAATLGIVVGCFLCWLPPFLVMPIGSFPP	
	301	360
Gerbil	DFKPPETVFKIVFWLGYLNSCINPIIYPCSSQEFKAPQNVLRQCLRRPSSKHALGYT	
Rat	DFKPPETVFKIVFWLGYLNSCINPIIYPCSSQEFKAPQNVLRQCLRRQSSKHALGYT	
Mouse	NFKPPETVFKIVFWLGYLNSCINPIIYPCSSQEFKAPQNVLRQCLRRQSSKHALGYT	
Human	DFKPPETVFKIVFWLGYLNSCINPIIYPCSSQEFKAPQNVLRQCLRRQSSKHALGYT	
	361	420
Gerbil	LHPPSQAVEGQHRGMVRI PVGSGETFFYKISKADGVCEWKLSSMPQGSARITVPKDQSSAC	
Rat	LHPPSQALEGQHRDMVRI PVGSGETFFYKISKTDGVCEWKFSSMPQGSARITVPKDQSSAC	
Mouse	LHPPSQAVEGQHRGMVRI PVGSGETFFYKISKTDGVCEWKFSSMPQGSARITVPKDQSSAC	
Human	LHPPSQAVEGQHKDMVRI PVGSGRETFFYKISKTDGVCEWKFSSMPQGSARITVSKDQSSC	
	421	467
Gerbil	TTARVRSKSFQVCCCVGSSTPSLEENHQVPTIKIHTISLGENGEV	
Rat	TTARVRSKSFQVCCCVGSSTPSLEENHQVPTIKIHTISLGENGEV	
Mouse	TTARVRSKSFQVCCCVGSSTPSLEENHQVPTIKIHTISLGENGEV	
Human	TTARVRSKSFQVCCCVGSSTPSLEENHQVPTIKIHTISLGENGEV	

Proposed membrane spanning domains are underlined. Conserved amino acids are shaded.

Table 4

Comparison of affinities of assorted α_1 -adrenergic receptor antagonist drugs in various species.

Drug	Gerbil	Human	Mouse	Rat
Prazosin	0.89±0.09	0.13 – 0.21	0.042 – 0.48	0.26 – 0.69
WB-4101	1.0±0.1	0.23 – 1.3	0.72 – 1.4	0.063 – 2.1
5-methylurapidil	4.9±1	3.2 – 30	0.24 – 2.8	0.16 – 3.2
BMY-7378	260±60	250	95 – 720	160 – 260

Affinities are expressed as K_i values in nM. Affinities of drugs for the gerbil α_1 -adrenergic receptor are mean values $\pm$ S.E. of at least 3 separate experiments on tissues from individual animals. For human, mouse and rat, affinities are ranges of values from a collection of radioligand binding studies of recombinant α_1 - and native endogenous α_1 -adrenergic receptors (Bockman et al., 2004; Bruchas et al., 2008; Goetz et al., 1995; Hirasawa et al., 1993; Lomasney et al., 1991; Morrow et al., 1986; Patel et al., 2001; Porter et al., 1992; Schwinn et al., 1995; Waugh et al., 2001; Weinberg et al., 1994; Xiao et al., 1998; Yang et al., 1998).