Species-Specific Pharmacological Properties of Human \(\alpha_{2A}\)-Adrenoceptors

Gerhard J. Molderings, Heinz Bönisch, Michael Brüss, James Likungu, Manfred Göthert

Abstract—On the basis of data obtained in rabbits, the imidazoline receptor ligand rilmenidine has been suggested to decrease blood pressure in humans by activating central \(\alpha_{2A}\)-adrenoceptors. A prerequisite for this hypothesis was the unproved assumption that rabbit and human \(\alpha_{2A}\)-adrenoceptors are equally activated by rilmenidine. Because \(\alpha_{2A}\)-adrenoceptors in the brain and on cardiovascular sympathetic nerve terminals are identical, the latter were used as a model for the former to confirm or disprove this assumption. Human atrial appendages and rabbit pulmonary arteries were used to determine the potencies of \(\alpha_{2}\)-adrenoceptor agonists in inhibiting the electrically (2 Hz) evoked \(^{[3}\text{H}]\)norepinephrine release and of antagonists in counteracting the \(\alpha_{2}\)-adrenoceptor–mediated inhibition induced by moxonidine. In the rabbit pulmonary artery, rilmenidine and oxymetazoline are potent full agonists, whereas in the human atrial appendages they are antagonists at the \(\alpha_{2}\)-autoreceptors, sharing this property with rauwolscine, phentolamine, and idazoxan. In contrast, prazosin is ineffective. In addition, a partial nucleotide and amino acid sequence of the rabbit \(\alpha_{2A}\)-adrenoceptor (a region known to substantially influence the pharmacological characteristics of the \(\alpha_{2}\)-adrenoceptor) revealed marked differences between the rabbit and the human \(\alpha_{2A}\)-adrenoceptor. The sympathetic nerves of both the human atrial appendages and rabbit pulmonary artery are endowed with \(\alpha_{2A}\)-autoreceptors, at which, however, both rilmenidine and oxymetazoline exhibit different properties (agonism and antagonism, respectively). The antagonistic property of rilmenidine at human \(\alpha_{2A}\)-adrenoceptors indicates that in contrast to the suggestion based on rabbit data, the hypotensive property of the drug in humans is not due to activation of \(\alpha_{2A}\)-adrenoceptors but other, presumably I1-imidazoline receptors, are probably involved. (Hypertension. 2000;36:405-410.)

Key Words: receptors, adrenergic, alpha | human | norepinephrine | rabbits | rilmenidine

Four subtypes of \(\alpha_{2}\)-adrenoceptors, \(\alpha_{2A}, \alpha_{2B}, \alpha_{2C},\) and \(\alpha_{2D}\) have been defined on the basis of their pharmacological properties. 1 The \(\alpha_{2A}\)-adrenoceptors (present in humans, pigs, and rabbits) and \(\alpha_{2D}\)-adrenoceptors (in rats, mice, guinea pigs, and cattle) represent species homologs of the same receptor. 1,2 Although molecular genetics indicate that \(\alpha_{2A}\) and \(\alpha_{2D}\)-adrenoceptors are structurally very similar among the species, both recombinant and native receptors exhibit different pharmacological properties. Differences in potency and intrinsic activity of \(\alpha\)-adrenoceptor ligands even exist between the \(\alpha_{2A}\)-adrenoceptors of different species. For example, oxymetazoline behaved as an agonist at the \(\alpha_{2A}\)-adrenoceptors of the rabbit pulmonary artery but as an antagonist at the \(\alpha_{2A}\)-adrenoceptors of the human saphenous vein. 2 Therefore, it is not possible to reliably predict the action of a given \(\alpha\)-adrenoceptor ligand at human \(\alpha_{2A}\)-adrenoceptors on the basis of data obtained for \(\alpha_{2A}\)-adrenoceptors of other species, in particular the rabbit. Nevertheless, on the basis of results obtained in rabbits, rilmenidine has been suggested to decrease blood pressure in humans by activating \(\alpha_{2A}\)-adrenoceptors in the rostral ventrolateral medulla 3,4 without the necessity to postulate an action at I1-imidazoline receptors. 5,6 A prerequisite for this hypothesis was the assumption that rilmenidine activates not only rabbit but also human \(\alpha_{2A}\)-adrenoceptors, which, however, has not yet been proved; in contrast, rilmenidine exhibited no intrinsic activity (and only very low affinity) at recombinant human \(\alpha_{2A}\)-adrenoceptors. 7 Because native \(\alpha_{2A}\)-adrenoceptors in the brain and on cardiovascular sympathetic nerve terminals (inhibitory presynaptic \(\alpha_{2}\)-autoreceptors) are identical, the latter can be used as a model for the former.

Whereas it is generally accepted that the presynaptic \(\alpha_{2}\)-autoreceptors in the rabbit cardiovascular system belong to the \(\alpha_{2A}\)-subtype, their subclassification in human cardiovascular tissue is equivocal: In the human saphenous vein, the presynaptic \(\alpha_{2}\)-autoreceptor was found to be of the \(\alpha_{2A}\)-subtype; 2 but in the human heart, it was suggested to belong to the \(\alpha_{2C}\)-subtype; 3 if true, the latter would be an exception.

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to the general rule that the presynaptic α₂ₐ-autoreceptor is of the α₂A/D-subtype. Because the discrepancies in the subclassification of the presynaptic α₂ₐ-autoreceptors in human cardiovascular tissue may be due to misinterpretation of pharmacological data, the first aim of the present study was to re-investigate whether or not the presynaptic α₂ₐ-autoreceptor in the human heart actually belongs to the α₂ₐ-subtype. For this purpose, α₂ₐ-autoreceptor ligands that clearly discriminate between α₂ₐ and α₂ₐ-autoreceptors such as prazosin have been applied. On the basis of the results obtained in this context, it should be examined whether native resemble recombinant human α₂ₐ-autoreceptors in that rilmenidine exhibits no intrinsic activity; in vivo, this should result in antagonism instead of the agonism shown for the rabbit α₂ₐ-autoreceptors. The characterization of rilmenidine as an antagonist at human α₂ₐ-autoreceptors would be of high clinical significance because it would exclude the possibility that the hypotensive effect of this drug is due to central α₂ₐ-autoreceptor activation. In contrast, this would provide strong indirect evidence for an involvement of other receptors, in particular L₁-imidazoline receptors, in the antihypertensive effect of rilmenidine. Second, to investigate whether differences in the pharmacological characteristics of the α₂ₐ-autoreceptors in rabbit pulmonary artery and human atrial appendages were related to differences in molecular structure of these receptors, a partial nucleotide sequence of the rabbit α₂ₐ-autoreceptor assumed to be relevant for the pharmacological character of the adrenoceptor was determined and compared with those of the human α₂ₐ-autoreceptor.

Methods

Superfusion Experiments

Segments of pulmonary artery were excised from male White New Zealand rabbits, and segments of macroscopically normal human right atrial appendages were obtained from normotensive 35- to 70-year-old male or female patients undergoing open heart surgery. The atrial appendages were routinely removed for cannulation of the right atria. The patients were not treated with adrenoceptor agonists or antagonists or with drugs influencing the storage or release of norepinephrine. The study was approved by the local ethics committee. The experiments with animal tissue were conducted in accordance with the German Law for Care and Use of Laboratory Animals. Tissue preparation and the protocol of the superfusion experiments with rabbit pulmonary artery and human atrial appendages has been described in detail previously. The electrically (2 Hz or in a few experiments 6 Hz) evoked tritium overflow from the superfused preparations preincubated with [³H]norepinephrine was determined, which in the presence of blockade of neuronal and extraneuronal uptake reflects the action potential-induced release of tritiated and endogenous norepinephrine from the sympathetic neurons. Results are given as mean±SEM. Student’s t test for unpaired data was used for comparison of mean values. As an estimate of potency, the concentration that reduces evoked tritium overflow by 35% (IC₃₅) was determined by interpolation between the 2 nearest points of the concentration-response curve. pIC₃₅ are the negative logarithms of these concentrations. Apparent pA₂ values at the level of the IC₃₅ were determined according to formula (4) of Furchgott. Cloning and Sequencing of Rabbit α₂ₐ-Autoreceptor

Genomic DNA was isolated from brain cortex of White New Zealand rabbits. The solution then was extracted twice with phenol/chloroform/isoamylalcohol (25:24:1). The aqueous phase was purified with a Chroma Spin 30 column (Clontech) according to the manufacturer. The purified DNA was used as template for subsequent polymerase chain reaction (PCR) amplification of the α₂ₐ-adrenoceptor DNA under the following conditions: primer sequences were chosen for the 4th (sense primer: 5′GGAAATCTCATCTCTTCCGCC(A/G)/CTCAT3′) and 5th (antisense primer: 5′GGAC- TAGTCACA(C/A)TAGACCAGGATCATGAT3′) transmembrane regions. PCR was performed in a total of 100 μL containing 15 mmol/L primer (each), 5 U Taq DNA Polymerase (Gibco), 2 mmol/L MgCl₂, 200 μmol/L dNTPs (each), 10 μL 10× Taq-Buffer (Gibco), and 5 μL template DNA. PCR was performed for 37 cycles (94°C, 1 minute; 64°C, 1 minute; 72°C, 3 minutes). PCR products were separated by agarose gel electrophoresis, and the band of interest was cut off the gel, purified with “GeneElute” columns (Supelco), ligated into the “TA-cloning” vector pCRII (Invitrogen) and transformed into Escherichia coli InVa’ (Invitrogen). The α₂ₐ-adrenoceptor DNA was sequenced on both strands by the dideoxynucleotide chain termination method and the use of the Sequenase 2.0 Kit (Amersham) and [¹⁻³⁵S]⁰-dATP. Sequencing products were run on 6% denaturing polyacrylamide gels and bands were visualized by autoradiography with XAR-5 (Kodak) films overnight.

Drugs used were (-)-(2S,55,6S)-¹Hnorepinephrine (specific activity 55 Ci/mmol, New England Nuclear); prazosin hydrochloride, cocaine hydrochloride, corticosterone, rauwolscine hydrochloride (Sigma); oxymetazoline hydrochloride (Merck); desipramine hydrochloride, phenolamine hydrochloride (CIBA-GEIGY); moxonidine (Beiersdorf-Lilly); (±)-idoazoxan hydrochloride (Reckitt and Colman); methiothepin maleate (Hoffmann-La Roche); and rilmenidine dihydrogenphosphate (Servier). Drugs were dissolved in saline except for corticosterone, which was dissolved in propaniol-1,2. The vehicles had no effect on basal tritium efflux or evoked tritium overflow.

Results

Superfusion Experiments

Basal Tritium Efflux

Under control conditions, basal tritium efflux from strips of the human atrial appendages and rabbit pulmonary artery preincubated with [¹H]norepinephrine decreased with time (not shown; for details, see References 2 and 14). Basal tritium efflux in the presence of the α₂ₐ-adrenoceptor ligands and methiothepin did not significantly differ from that in the absence of these drugs (not shown).

Electrically Evoked Tritium Overflow Under Control Conditions

When no test drug was administered throughout superfusion, the tritium overflow evoked by the reference stimulation period S₂ at 2 Hz (standard frequency) amounted to 11.6±1.9 nCi (corresponding to 0.90±0.13% of tissue tritium; n=20) in human atrial appendages and to 7.6±1.1 nCi (corresponding to 0.52±0.05% of tissue tritium; n=21) in rabbit pulmonary artery. In the control experiments (absence of αₐ-adrenoceptor agonists), the evoked tritium overflow in both tissues either slightly decreased from S₂ to S₃ or remained approximately constant, as reflected by S₃/S₂ ratios close to unity (data not shown; for details, see legend to the Figure and References 2 and 14).

Effects of α₂ₐ-Autoreceptor Ligands on Evoked Tritium Overflow

Human Atrial Appendages

Moxonidine inhibited the electrically evoked tritium overflow (Figure 1A and 1B and Table 1), whereas rilmenidine failed to
were present from 14 minutes before S1 until end of experiments. All inhibitory effects of moxonidine, rilmenidine, and experiments in presence of 0.1 μmol/L rauwolscine ( ), 0.1 μmol/L idazoxan ( ), 0.3 μmol/L phentolamine ( ), and 1 μmol/L prazosin ( ). Values are mean±SEM of 5 to 11 experiments.

B. Effect of moxonidine on electrically evoked tritium overflow from human atrial appendages and interaction with oxymetazoline and rilmenidine. Shown are experiments without oxymetazoline and rilmenidine ( ) and experiments in presence of 0.1 μmol/L oxymetazoline ( ) or 1 μmol/L rilmenidine ( ). Mean±SEM of 6 to 8 experiments. C, Effects of rilmenidine and (D) oxymetazoline on electrically evoked tritium overflow from human atrial appendages ( ) and rabbit pulmonary artery ( ). Mean±SEM of 6 to 10 experiments. Five periods of transmural electrical stimulation (2 Hz: S1-S5) were applied. Agonists in A through D were applied at increasing concentrations from 12 minutes before until 20 minutes after onset of S5, S6, and S7. Interacting drugs were present from 14 minutes before S5 until end of experiments. Ordinate: S3/S2, S4/S2, and S5/S2 overflow ratios, expressed as percentage of ratios in corresponding control experiments. All inhibitory effects of moxonidine, rilmenidine, and oxymetazoline by >30% were significantly different from corresponding controls (at least P<0.05; for the sake of clarity, asterisks indicating levels of significance have been omitted), +P<0.05 (compared with effect of respective moxonidine concentration in absence of interacting drugs).

Figure 1. A, Effect of moxonidine on electrically evoked tritium overflow from human atrial appendages and interaction with α2-adrenoceptor antagonists. Shown are experiments without α2-adrenoceptor antagonist ( ) and experiments in presence of 0.1 μmol/L rauwolscine ( ), 0.1 μmol/L idazoxan ( ), 0.3 μmol/L phentolamine ( ), and 1 μmol/L prazosin ( ). Values are mean±SEM of 5 to 11 experiments. B, Effect of moxonidine on electrically evoked tritium overflow from human atrial appendages and interaction with oxymetazoline and rilmenidine. Shown are experiments in absence of oxymetazoline and rilmenidine ( ) and experiments in presence of 0.1 μmol/L oxymetazoline ( ) or 1 μmol/L rilmenidine ( ). Mean±SEM of 6 to 8 experiments. C, Effects of rilmenidine and (D) oxymetazoline on electrically evoked tritium overflow from human atrial appendages ( ) and rabbit pulmonary artery ( ). Mean±SEM of 6 to 10 experiments. Five periods of transmural electrical stimulation (2 Hz: S1-S5) were applied. Agonists in A through D were applied at increasing concentrations from 12 minutes before until 20 minutes after onset of S5, S6, and S7. Interacting drugs were present from 14 minutes before S5 until end of experiments. Ordinate: S3/S2, S4/S2, and S5/S2 overflow ratios, expressed as percentage of ratios in corresponding control experiments. All inhibitory effects of moxonidine, rilmenidine, and oxymetazoline by >30% were significantly different from corresponding controls (at least P<0.05; for the sake of clarity, asterisks indicating levels of significance have been omitted), +P<0.05 (compared with effect of respective moxonidine concentration in absence of interacting drugs).

Rabbit Pulmonary Artery
Rilmenidine and oxymetazoline inhibited the electrically evoked tritium overflow (Figure 1C and Table 1). At the highest concentration investigated, the compounds inhibited the electrically evoked tritium overflow by ≈70%.

Cloning and Sequencing of Rabbit α2A-Adrenoceptor
The nucleotide and amino acid sequence data for the rabbit clone (present study) and the amino acid sequence of the human, porcine, rat, and mouse clones (found by BLAST search in protein databases at http://www.ncbi.nlm.nih.gov/ cgi-bin/BLAST/nph-newblast) are given in Table 2. The sequence for the rabbit clone is homologous to the other α2A/D subtypes and shows a cysteine (TGC) at position 201. As compared with the human sequence in this region, the rabbit clone exhibits a 75% homology in the deduced amino acid sequence and a 69% homology in the nucleotide sequence (Table 2). The rabbit amino acid sequence differs from the human sequence in positions 174, 181, 184, 185, 186, 199, 202, and 207; only the changes in position 174 and 199 are conservative. Moreover, there is a deletion at the human amino acid positions 182 and 183.

Discussion
Subclassification of Presynaptic α2-Autoreceptor in Human Heart Atrium
In a first investigation designed to subclassify the α2-autoreceptors in human atrial appendages, prazosin (which in addition to its antagonistic property at α1-adrenoceptors is a potent antagonist at α2A and α2C-adrenoceptors) failed to antagonize the inhibitory effect of the full α2-adrenoceptor agonist UK14304 on electrically evoked norepinephrine release under experimental conditions comparable to the present ones (see Figure 4 in Reference 16). However, at modified stimulation parameters (5 trains of 10 pulses at 100 Hz), a slight nonparallel rightward shift of the concentration-response curve of UK14304 by 0.1 μmol/L prazosin was found that was overinterpreted as a hint at an antagonism at α2-α-adrenoceptors.8 This conclusion appeared at that time to be supported by a comparison with the α2-autoreceptors in the human kidney, which had been suggested to be of the α2C-subtype.17 However, in a recent reinvestigation by the same group, the presynaptic α2-autoreceptors in the human kidney could be unequivocally subclassified as α2A,9 which is in agreement with the subclassification of the α2-autoreceptors in human saphenous vein2 and brain.18 This conforms to the rule that α2-autoreceptors belong predominantly to the genetic α2A/D-subtype of the α2-adrenoceptor.9
In the present experiments, 5 lines of evidence argue in favor of an identity of the \( \alpha_2 \)-autoreceptors with the \( \alpha_2A \)-subtype and against an identity with the \( \alpha_2B \) or \( \alpha_2C \)-subtypes: (1) Prazosin (1 \( \mu \)mol/L) did not counteract the moxonidine-induced inhibition of evoked norepinephrine release, although this concentration is 11 and 32 times higher than its binding affinity at recombinant \( \alpha_2B \)- and \( \alpha_2C \)-adrenoceptors (Table 1). Prazosin concentrations \( \geq 1 \mu \)mol/L could not be applied because they produced a marked increase in basal tritium efflux. (2) The lack of intrinsic activity of oxymetazoline in the native human atrial appendages would be compatible with both an \( \alpha_2A \) and \( \alpha_2C \) character. However, the relatively high potency of oxymetazoline in shifting the concentration-response curve for moxonidine to the right (ie, in acting as an antagonist: apparent \( pA_2 \) value 7.77) argues against an \( \alpha_2C \) as well as an \( \alpha_2B \) character of these receptors. (3) The affinity ratio of oxymetazoline/rauwolscine is particularly suitable to discriminate between the \( \alpha_2 \)-adrenoceptor subtypes (for details see Reference 9). In human atrial appendages, this ratio (3.6) is close to that for recombinant \( \alpha_2A \)-sites (4.2) but distinctly different from the ratios for recombinant \( \alpha_2B \)-sites (3.56) and \( \alpha_2C \)-sites (3.17). (4) When the potencies of the compounds acting as antagonists at the presynaptic \( \alpha_2 \)-adrenoceptors of the human heart (Table 1) were compared with their affinities for human recombinant \( \alpha_{2A,B,C} \)-adrenoceptors, there was a significant correlation for \( \alpha_2A \)-adrenoceptors (\( r=0.93; \) \( P<0.03; \) regression line almost identical to the line of identity) but not for \( \alpha_2B \) and \( \alpha_2C \)-adrenoceptors. (5) Moxonidine was recently shown to be devoid of agonistic activity at human recombinant \( \alpha_{2A,B,C} \)-adrenoceptors, whereas it acted as a full agonist at the human recombinant \( \alpha_2A \)-adrenoceptor (Table 1 and Reference 19).

### TABLE 1. Agonistic and Antagonistic Potencies

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<th>( pIC_{35%} ), Rabbits</th>
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<th>( h\alpha_{2B} )</th>
<th>( h\alpha_{2C} )</th>
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\( pA_2 \) Values

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A agonistic and antagonistic potencies of \( \alpha_2 \)-adrenoceptor ligands at presynaptic \( \alpha_2 \)-autoreceptors in human atrial appendages and rabbit pulmonary artery (\( pIC_{35\%} \), and apparent \( pA_2 \) values, respectively) as well as \( \alpha_2 \)-adrenoceptor radioligand binding affinity (\( pK_i \)) and intrinsic activity (IA) derived from \( [\text{35S}] \text{GTP}-\gamma-S \) binding at human \( \alpha_{2A,B,C} \)-adrenoceptors expressed in HEK293 cells.7 Moxonidine binding affinity and intrinsic activity at human recombinant \( \alpha_{2A,B,C} \)-adrenoceptors are taken from Reference 19. 0 indicates no intrinsic activity.

*From Reference 25.
†From Reference 26.

In the present experiments, 5 lines of evidence argue in favor of an identity of the \( \alpha_2 \)-autoreceptors with the \( \alpha_2A \)-subtype and against an identity with the \( \alpha_2B \) or \( \alpha_2C \)-subtypes: (1) Prazosin (1 \( \mu \)mol/L) did not counteract the moxonidine-induced inhibition of evoked norepinephrine release, although this concentration is 11 and 32 times higher than its binding affinity at recombinant \( \alpha_2B \)- and \( \alpha_2C \)-adrenoceptors (Table 1). Prazosin concentrations \( \geq 1 \mu \)mol/L could not be applied because they produced a marked increase in basal tritium efflux. (2) The lack of intrinsic activity of oxymetazoline in the native human atrial appendages would be compatible with both an \( \alpha_2A \) and \( \alpha_2C \) character. However, the relatively high potency of oxymetazoline in shifting the concentration-response curve for moxonidine to the right (ie, in acting as an antagonist: apparent \( pA_2 \) value 7.77) argues against an \( \alpha_2C \) as well as an \( \alpha_2B \) character of these receptors. (3) The affinity ratio of oxymetazoline/rauwolscine is particularly suitable to discriminate between the \( \alpha_2 \)-adrenoceptor subtypes (for details see Reference 9). In human atrial appendages, this ratio (3.6) is close to that for recombinant \( \alpha_2A \)-sites (4.2) but distinctly different from the ratios for recombinant \( \alpha_2B \)-sites (3.56) and \( \alpha_2C \)-sites (3.17). (4) When the potencies of the compounds acting as antagonists at the presynaptic \( \alpha_2 \)-adrenoceptors of the human heart (Table 1) were compared with their affinities for human recombinant \( \alpha_2A \)-, \( \alpha_2B \)-, and \( \alpha_2C \)-adrenoceptors, there was a significant correlation for \( \alpha_2A \)-adrenoceptors (\( r=0.93; \) \( P<0.03; \) regression line almost identical to the line of identity) but not for \( \alpha_2B \) and \( \alpha_2C \)-adrenoceptors. (5) Moxonidine was recently shown to be devoid of agonistic activity at human recombinant \( \alpha_2B \) and \( \alpha_2C \)-adrenoceptors, whereas it acted as a full agonist at the human recombinant \( \alpha_2A \)-adrenoceptor (Table 1 and Reference 19).

### TABLE 2. Nucleotide and Amino Acid Sequence and Homologies

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\( \alpha_{2A} \) (rabbit)  
\( \alpha_{2A} \) (human)  
\( \alpha_{2A} \) (pig)  
\( \alpha_{2A} \) (rat)  
\( \alpha_{2A} \) (mouse)

A, Nucleotide and amino acid sequence from 4th to 5th transmembrane regions of \( \alpha_{2A} \)-adrenoceptor of rabbit; and B, homologies of deduced amino acid sequences between rabbit, human, porcine, rat, and mouse \( \alpha_{2A,B} \)-adrenoceptors. 0 indicates amino acids conserved with human \( \alpha_{2A,B} \)-adrenoceptors.

*Deletions.
Different Pharmacological Properties of Human and Rabbit $\alpha_2$-Autoreceptors

Although both $\alpha_2$-autoreceptors in human (Reference 2; present study) and rabbit blood vessels can be classified as $\alpha_2A$-adrenoceptors on the basis of the effects of $\alpha_2$-adrenoceptor antagonists, the results of the present study highlight pronounced differences in the effects of rilmenidine and oxymetazoline. They were full agonists at rabbit $\alpha_2$-autoreceptors but they antagonized the inhibitory effect of moxonidine on evoked norepinephrine release from human atrial appendages. Their antagonistic potency is very similar to their affinity determined in radioligand binding studies (Table 1). At a stimulation frequency of 6 Hz, at which the concentration of the endogenous norepinephrine in the sympathetic cleft is much higher, leading to a more pronounced activation of the $\alpha_2A$-autoreceptors than at 2 Hz, rilmenidine enhanced evoked $[\text{H}]$-norepinephrine release; in other words, it again exhibited the typical behavior of an antagonist at $\alpha_2A$-autoreceptors in that it disinhibited release. Interestingly, it has recently been reported that rilmenidine was also devoid of agonistic activity at the $\alpha_2A$-adrenoceptors in porcine tail artery and urinary bladder, which are structurally most similar to the human $\alpha_2A$-adrenoceptors (Table 2).

Relation Between Pharmacological Subclassification and Genetic Encoding of the Receptor

The construction, expression, and pharmacological characterization of chimeric mouse $\alpha_2F$/human $\alpha_2A$-adrenoceptors and mutant $\alpha_2A$-adrenoceptors led to the assumption that the amino acid sequence spanning the fifth transmembrane region, in particular the cysteine residue at position 201, might determine the pharmacological properties of the $\alpha_2A$-adrenoceptor. In fact, the human and porcine genes for the $\alpha_2A$-adrenoceptors are highly conserved amino acids and a deletion of 2 amino acids (4 changes of $\alpha_2A$-adrenoceptors) and a deletion of 2 amino acids (4 changes of $\alpha_2D$/human $\alpha_2A$-adrenoceptors) (Table 2). Taken together, our observations of different pharmacological characteristics of human and rabbit $\alpha_2A$-adrenoceptors are reflected by substantial differences between both receptors in the nucleotide and amino acid sequence.

Implications for the Anti hypertensive Effect of Rilmenidine

As outlined in the introduction, rilmenidine has been suggested to decrease blood pressure in humans by activating $\alpha_2$-adrenoceptors in the rostral ventrolateral medulla, which belong to the $\alpha_2C$-subtype. However, in view of the antagonistic property of rilmenidine at human $\alpha_2A$-adrenoceptors found here, this possibility must be excluded. Therefore, the hypotensive effect of the drug must be due to another mechanism, probably to an activation of $\alpha_1$-imidazoline receptors (for review, see References 23 and 24).

Acknowledgments

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References

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