

COMPOUNDS USEFUL FOR THE TREATMENT OR PREVENTION OF A DISEASE MEDIATED BY THE ALPHA-2B-ADRENOCEPTOR

The present invention relates to the use of selective alpha-2B-adrenoceptor antagonists for the manufacture of a pharmaceutical preparation useful for the treatment or prevention of diseases mediated by the alpha-2B-adrenoceptor in mammals. The present invention also relates to a method for the treatment or prevention of diseases mediated by the alpha-2B-adrenoceptor in mammals, by administering to said mammal said selective alpha-2B-adrenoceptor antagonist.

BACKGROUND OF THE INVENTION

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated by reference.

The selective alpha-2B-adrenoceptor antagonists shown in Scheme I below are all previously known. The inventors obtained the compounds A (ordering No AE-848/34956037), C (ordering No AF-399/36012031) and D (ordering No AH-034/34347043) from SPECS and BioSPECS B.V., Fleminglaan 16, 2289 CP Rijswijk, The Netherlands. The compounds B (ordering No 653716) and E (ordering No 569063) were supplied by ChemBridge Corporation, 16981 Via Tazon, Suite G, San Diego CA 92127.

It is known that alpha-2B-adrenoceptors mediate vascular contractions. Therefore, alpha-2B-antagonists are useful in the treatment or prevention of diseases involving vascular contraction. It has also been found that there is a genetic polymorphism in the alpha-2B-adrenoceptor gene at certain individuals. It has been observed that the alpha-2B-adrenoceptor protein at some subjects has a deletion of 3 glutamates from the glutamic acid repeat element of 12 glutamates (amino acids 297-309), in an acid

trech of 17 amino acids, located in the third intracellular loop of the receptor polypeptide (Heinonen et al., 1999).

OBJECTS AND SUMMARY OF THE INVENTION

It has now been found that the compounds selected from the group consisting of compound A, B, C, D and E, the formulae of which are disclosed in Scheme I, are selective alpha-2B-adrenoceptor antagonists.

Thus, this invention relates to the use of a selective alpha-2B-adrenoceptor antagonist for the manufacture of a pharmaceutical preparation useful for the treatment or prevention of a disease mediated by the alpha-2B-adrenoceptor in a mammal. According to the invention, wherein said antagonist is a compound selected from the group consisting of compound A, B, C, D and E disclosed in Scheme I, or a pharmaceutically acceptable salt of said compound.

This invention relates also to a method for the treatment or prevention of a disease mediated by the alpha-2B-adrenoceptor in a mammal, said method comprising administering to said mammal an effective amount of a selective alpha-2B-adrenoceptor antagonist, wherein said antagonist is a compound selected from the group consisting of compound A, B, C, D and E disclosed in Scheme I, or a pharmaceutically acceptable salt of said compound.

DETAILED DESCRIPTION OF THE INVENTION

Alpha-2B-adrenoceptor antagonists are useful in the treatment and/or prevention of many diseases.

Individuals having a deletion in the alpha-2B-adrenoceptor protein (Heinonen et al., 1999), particularly the deletion/deletion genotype (D/D genotype) is an important

target group which benefits from administration of selective alpha-2B-adrenoceptor antagonists.

It has been found that in a population-based cohort of Finnish middle-aged men that subjects with a D/D genotype of the alpha-2B-adrenoceptor gene have a significantly elevated risk for acute myocardial infarction (AMI) in a five-year follow-up study. The risk for AMI was increased in subjects who had no previously diagnosed coronary heart disease (CHD) at the study outset. Therefore, it has been postulated that the D/D genotype is related to an impaired capacity to down-regulate alpha-2B-adrenoceptor function during sustained receptor activation. Therefore, alpha-2B-adrenoceptors are believed to be involved in the pathogenesis of a significant fraction of all cases of AMI, especially in subjects with the D/D genotype, but also in I/D and I/I subjects (I means "insertion" and stands for the "normal" allele).

The alpha-2B-adrenoceptor antagonists as disclosed in this invention would be particularly useful in the treatment or prevention of coronary heart diseases. As examples can be mentioned

a) Acute AMI

If alpha-2B-adrenoceptor dependent vasoconstriction is a causative factor in some cases of AMI, then antagonism of these receptors should restore coronary circulation and reduce the ischemic myocardial damage.

b) Unstable angina pectoris

An alpha-2B-adrenoceptor antagonist will relieve the vasoconstrictive component in the sustained ischemic episode, thus alleviating the symptoms and preventing AMI.

c) Prinzmetal's variant form of angina pectoris

Vasoconstriction is a key factor in the pathogenesis of Prinzmetal's angina, and an alpha-2B- adrenoceptor antagonist may resolve and prevent attacks.

d) Other forms of chronic angina pectoris and CHD

An alpha-2B-adrenoceptor antagonist will help to alleviate the vasoconstrictive component in all types of CHD, providing both symptomatic relief and protection from AMI. A general reduction in vascular tone will contribute to this by reducing venous return, cardiac workload and oxygen consumption (a nitrate-type effect; see below).

e) Prevention of restenosis after coronary angioplasty in cases where vasoconstriction plays a role in restenosis.

Furthermore, the alpha-2B-adrenoceptor antagonists as disclosed in this invention would be useful in the treatment or prevention of essential hypertension, especially in subjects with increased sympathetic activity and a hyperdynamic circulatory system.

In the study mentioned above, the D/D variant of the alpha-2B-adrenoceptor gene was not clearly associated with blood pressure. The inventors believe that this was due to two main factors, 1) antihypertensive treatment, and 2) complex regulation of systemic blood pressure. In another study (Heinonen et al.), it was observed that the D/D genotype was associated with reduced basal metabolic rate and reduced heart rate. These associations probably reflect increased vascular resistance in these subjects.

In transgenic mice with targeted inactivation of the alpha-2B-adrenoceptor gene, intravenously administered alpha-2-adrenoceptor agonists fail to induce the characteristic blood pressure elevation which is seen in normal animals and also in humans after large doses of such drugs (Link et al., 1996). The hypotensive effect of these drugs was markedly accentuated. This demonstrates that alpha-2B-adrenoceptors mediate vascular contraction. Thus, an antagonist should reduce blood pressure. This effect has not been seen with alpha-2B-nonselective alpha-2-adrenoceptor antagonists, because antagonism of alpha-2A-adrenoceptors increases sympathetic outflow, cardiac output and blood pressure. In mice with dysfunctional alpha-2A-adrenoceptors, alpha-2-adrenoceptor agonists caused an accentuated hypertensive response and no hypotension (MacMillan et al., 1996).

An alpha-2B-adrenoceptor antagonist is postulated to have favourable effects in hypertensive subjects through their effects on renal function, muscle blood flow, and also on vascular resistance in other vascular beds. The anti-AMI effect of such a drug will be an additional benefit, as hypertension is a significant risk factor for AMI. This protection is due to three factors: 1) a reduction in systemic blood pressure, 2) decreased risk of coronary vasoconstriction, and 3) a nitrate-like effect on venous return, myocardial workload and oxygen consumption.

Moreover, the alpha-2B-adrenoceptor antagonists as disclosed in this invention would be useful in the treatment or prevention of other vascular diseases.

Specifically, benefits can be expected in the treatment or prevention of

- vasoconstriction and hypoxic brain damage subsequent to subarachnoid haemorrhage,
- migraine,
- Raynaud's disease and cold intolerance,
- pre-eclampsia,
- male erectile dysfunction, and
- obesity and the metabolic syndrome.

The last mentioned effect is due to the fact that reduced muscle blood flow and reduced basal metabolic rate contribute to the development of obesity and hypertension. An α -2B-adrenoceptor antagonist will, by increasing the muscle blood flow, increase energy expenditure and shift the caloric balance to a favourable direction.

The α -2B-adrenoceptor antagonists disclosed in this invention are also useful in anesthesia and analgesia to potentiate the clinical efficacy of α -2-adrenoceptor agonists which are not selective for the α -2B-adrenoceptor subtype. By blocking the vasoconstriction induced by these agonists, a simultaneously administered α -2B-adrenoceptor antagonist will allow the use of larger doses of said agonists, up to anesthetic dose levels which have not previously been possible in man, only in veterinary anesthetic practice.

EXPERIMENTAL SECTION

Binding affinity human α -2-adrenoceptor

The affinity of test compounds for the three human α_2 -adrenoceptor subtypes (α_{2A} , α_{2B} and α_{2C}) was determined in competition binding assays with ^3H -rauwolscine. The biological material for these experiments consisted of membranes from Shionogi S115 cells stably transfected with either of the three human α_2 subtypes (Marjamäki et al. 1992). Membrane (5-10 μg of total protein per sample) and 1 nM – 2 nM ^3H -rauwolscine (specific activity 78 Ci/mmol) were incubated in 50 mM KH_2PO_4 , pH 7.5 with 6 concentrations of the compounds. Each concentration was run in duplicate. Nonspecific binding was defined by 100 μM oxymetazoline and corresponded to 5 - 15% of total binding. After 30 min at room temperature, incubations were terminated by rapid vacuum filtration through GF/B glass fiber filter and three 5 ml washes with icecold incubation buffer. The filters were then

dried, impregnated with scintillate and their radioactivity was measured by scintillation counting. The analysis of the experiments was carried out by nonlinear least square curve fitting. Experimentally determined IC₅₀ values were converted to K_i's by making use of the Cheng-Prusoff equation (Cheng and Prusoff, 1973). Experiments were repeated a minimum of three times.

Table 1: Binding affinities on human α_2 -adrenoceptor subtypes

Data is presented as K_i's in nM (Mean $\pm$ SEM), n = 3 unless indicated otherwise

Compound	alpha-2A	alpha-2B	alpha-2C
A	4100 $\pm$ 200	30 $\pm$ 4	>4700
C	>30000 (n=2)	1860 (n=2)	>30000 (n=2)
D	>30000	530 $\pm$ 90	>30000
B	>30000	215 $\pm$ 60	>30000
E	>100000	2900 $\pm$ 300	>100000

Binding affinity rat cortical α_1 -adrenoceptor

The affinity for rat neocortical α_1 -adrenoceptors was determined in competition binding assays with ³H-prazosin. The biological material for these assays consisted of membranes from rat neocortex. Membrane suspensions (100-200 μ g of total protein per sample) and 0.2 nM-0.25nM of ³H-prazosin (specific activity 74 Ci/mmol) were incubated with 6 concentrations of compounds in a total volume of 0.25 ml (50 mM Tris pH 7.7 at 25°C). Each concentration was run in duplicate. Nonspecific binding was defined by 10 μ M phentolamine methanesulfonate and corresponded to 25-30 % of total binding. After 30 min at room temperature,

incubations was terminated by rapid filtration through GF/B glass-fiber filter mats and three washes with ice-cold 10 mM Tris (pH 7.7 at 4°C). After drying, a solid scintillate was melted onto the filter mats, and their radioactivity was measured by scintillation counting.

Result

At concentrations of up to 30 μ M, compound A caused insufficient displacement of 3 H-prazosin to allow the estimate of an IC_{50} value. It is therefore concluded that the IC_{50} and the K_i of compound A must be $>30\,000$ nM.

Antagonist activity on human α_2 -adrenoceptor subtypes

Antagonist potencies were determined as the ability of test compounds to competitively inhibit epinephrine-stimulated 35 S-GTP γ S binding to G proteins (Tian et al., 1993; Wieland and Jakobs, 1994; Jasper et al., 1998) in membranes of CHO cells stably transfected with one of the three human α_2 subtypes (Pohjanoksa et al., 1997; Marjamäki et al., 1998). Membranes (2-6 μ g of protein per sample) and 12 concentrations of test compound were preincubated for 30 min with a fixed concentration of epinephrine (5 μ M for α_{2A} , 15 μ M for α_{2B} , 5 μ M for α_{2C}) in 50 mM Tris, 5 mM $MgCl_2$, 150 mM NaCl, 1 mM DTT, 1 mM EDTA, 10 μ M GDP, 30 μ M ascorbic acid, pH 7.4 at room temperature. Binding of radiolabel was started by the addition of trace amounts of 35 S-GTP γ S (0.08 nM- 0.15 nM, specific activity 1250 Ci/mmol) to the incubation mixture. After an additional 60 min at room temperature, the incubation was terminated by rapid vacuum filtration through glass fiber filter. Filters were washed three times with 5 ml icecold wash buffer (20 mM Tris, 5 mM $MgCl_2$, 1 mM EDTA pH 7.4 at room temperature), dried and counted for radioactivity in a scintillation counter. Analysis of experiments was carried out by nonlinear least square fitting. Experiments were repeated at least three times.

Table 2: Antagonist effect of compound A and compound B on the human α_2 -adrenoceptor subtypes

Data is presented as KB's in nM (Mean $\pm$ SEM), n is a minimum of three experiments.

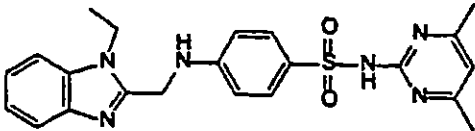
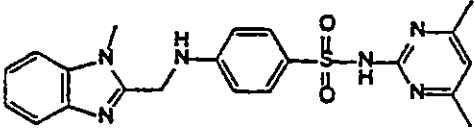
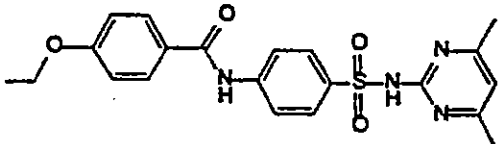
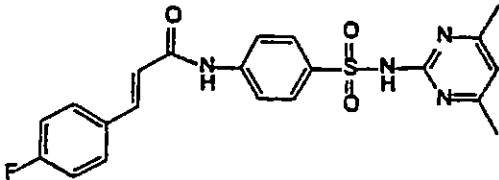
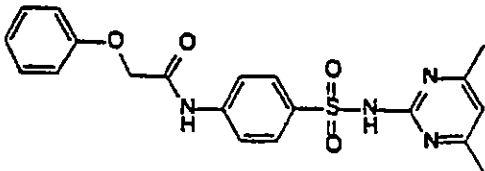
Compound	alpha-2A*	alpha-2B	alpha-2C*
A	2400 $\pm$ 700	73 $\pm$ 23	2400 $\pm$ 900
B	>10 000	250 $\pm$ 80	>10 000

* only incomplete dose-response curves could be obtained, KB numbers are minimum estimates

For the purpose of the invention, the alpha-2B-adrenoceptor antagonist as disclosed in Scheme I or its pharmaceutically acceptable salt can be administered by various routes. The suitable administration forms include, for example, oral formulations; parenteral injections including intravenous, intramuscular, intradermal and subcutaneous injections; transdermal or rectal administration forms. The required dosage of the compounds of the alpha-2B-adrenoceptor antagonist will vary with the particular condition being treated, the severity of the condition, the duration of the treatment, the administration route and the specific compound being employed. The suitable dose varies in the range 5 μ g to 100 mg per kg body weight and day for an adult person.

It will be appreciated that the methods of the present invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent for the specialist in the field that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

Scheme I

Compound
A

B

C

D

E


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CLAIMS

1. Use of a selective alpha-2B-adrenoceptor antagonist for the manufacture of a pharmaceutical preparation for the treatment or prevention of a disease mediated by the alpha-2B-adrenoceptor in a mammal, wherein said antagonist is a compound selected from the group consisting of compounds A, B, C, and E disclosed in Scheme I, or a pharmaceutically acceptable salt of said compound.
2. The use according to claim 1, wherein the disease is a coronary heart disease (CHD).
3. The use according to claim 2, wherein the disease is
 - acute myocardial infarction (AMI),
 - unstable angina pectoris,
 - Prinzmetal's variant form of angina pectoris,
 - other forms of chronic angina pectoris and CHD, or
 - restenosis after coronary angioplasty.
 -
4. The use according to claim 1, wherein the disease is essential hypertension.
5. The use according to claim 1, wherein the disease is a vascular disease, which is
 - vasoconstriction or hypoxic brain damage subsequent to subarachnoid hemorrhage,
 - migraine,
 - Raynaud's disease or cold intolerance,
 - pre-eclampsia,
 - male erectile dysfunction, or
 - obesity.

6. Use of a selective alpha-2B-adrenoceptor antagonist for the manufacture of a pharmaceutical preparation for potentiating the clinical efficacy of an anesthetic and/or analgetic alpha-2-adrenoceptor agonist, said agonist not being selective for the alpha-2B-adrenoceptor subtype.

7. A compound selected from the group consisting of compounds A, B, C and E disclosed in Scheme 1, or pharmaceutically acceptable salt of said compound for use as a medicament.