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(54) Title: GABA-C RECEPTOR AGONISTS OR ANTAGONISTS FOR USE IN THE TREATMENT OF VASCULAR DISEASES

(57) Abstract: The present invention relates to novel methods for regulation of blood flow, which may be used in particular in the treatment of retinal disorders that involve constriction or dilation of the retinal arterioles.



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GABA-C RECEPTOR AGONISTS OR ANTAGONISTS FOR USE IN THE TREATMENT OF VASCULAR DISEASES

Field of invention

- 5 The present invention relates to novel methods for regulation of blood flow, which may be used in particular in the treatment of retinal disorders that involve constriction or dilation of the retinal arterioles.

Background of invention

- 10 γ -Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian central nervous system, where it exerts its physiological effects through the ionotropic GABA_A and GABA_C receptors and the metabotropic GABA_B receptors. The GABA_A receptors are widely distributed in the CNS and involved in a wide variety of CNS functions. GABA_C receptors have also been identified in some CNS regions, where
- 15 they have been proposed to be involved in processes connected to regulation of sleep and cognition processes (Madsen et al., J Med Chem, 50:4147-4161; 2007). GABA_C receptors are predominately expressed in the retina and have primarily been thought to be implicated in visual processing.
- 20 Using GABA_C receptor rho(1) subunit-knockout mice, Zheng et al. (Exp Eye Res. 2010 May;90(5):634-40. Epub 2010 Mar 1) demonstrated that knockout of the retinal GABA_C rho(1) subunit in mice leads to changes in vascular permeability similar to the pathological changes induced by retinal hypoxic conditions. The authors conclude that the GABA_C rho(1) subunit plays a role in maintaining both homeostasis and balance of
- 25 retinal neurotransmitter function. Knockout of the retinal GABA_C rho(1)-subunit leads to changes in vascular permeability similar to the pathological changes induced by retinal hypoxic conditions.

- 30 The rho subunits of the GABA_C receptor are very different in mice compared to humans. The mice and the rat rho 1 subunits group together and diverges from the human rho 1 subunit in a dendrogram of their linkage based on analysis performed on the deduced amino acid sequence of each subunit.

- 35 Recent studies demonstrate that GABAergic activity elicits relaxation of retinal arterioles leading to an increase in blood flow. The mechanism underlying regulation of

arteriolar tone involves cellular components in both the vascular wall and the perivascular tissue. The relaxing effect of perivascular tissue on the porcine retinal arterioles has been examined *in vitro* and demonstrated that the relaxing effect is mimicked by N-methyl-D-aspartate (NMDA) and is blocked by prostaglandin synthesis inhibition (Holmgaard et al., Acta Ophthalmol; 86:26-33; 2008).

Summary of invention

The present invention demonstrates that agonists and antagonists of the GABA_C receptor have an effect on arteriole dilation and constriction respectively. Accordingly the invention presents novel methods for regulation of blood flow, which may be used in particular in the treatment of retinal disorders.

The present invention in one aspect relates to the use of GABA_C specific agonist and/or antagonist in regulation of vascular blood flow.

In another aspect, the invention relates to a method of regulating vascular blood flow in a subject by administering a therapeutically effective amount of at least one GABA_C specific agonist and/or antagonist.

In a further aspect, the invention relates to use of a GABA_C specific agonist or antagonist for the preparation of a medicament for regulation of vascular blood flow.

The inventor of the present invention has surprisingly found that GABA_C specific agonists and antagonists have an effect on the vasoconstriction and vasodilation of vascular arterioles in the presence of the perivascular tissue. GABA_A and GABA_B receptors are not involved in the regulation of vasoconstriction and vasodilation.

This shows that GABA_C specific agonists and antagonists may be used in treatment of diseases related to abnormal vascular blood flow. The regulation of vascular blood flow may be relevant in relation to treatment of a variety of disease and in one embodiment of the present invention the use is for treatment of retinal disorders.

The advantage of using GABA_C specific agonist and/or antagonist instead of e.g. GABA is that a more specific effect on the GABA_C receptor can be obtained thus avoiding any side effects caused by activation of GABA_A and GABA_B.

5 Description of Figures

Figure 1 shows the concentration dependent relaxation of retinal arterioles induced by GABA (units of concentration on the abscissa axis are 10^{-x} mol/liter) in the presence of perivascular retinal tissue and DL-APV in a concentration of 50 microM.

10 Figure 2A and 2B shows that none of the experiments showed vasodilation after addition of GABA or one of the GABA agonists to isolated retinal arterioles (dotted lines). Units of concentration on the abscissa axis are in 10^{-x} mol/liter.

Figure 2A shows that in the presence of the perivascular retinal tissue (solid lines) the GABA_A agonist Isoguvacine and the GABA_B agonist Baclofen had no effect on vascular tone, whereas the GABA_C agonist 5-methyl-imidazol-4-acetate induced a significant relaxation of the retinal arterioles at the three highest concentrations.

Figure 2B shows that in the presence of perivascular retinal tissue (solid lines) a blocking of the GABA_A receptor with picrotoxin (and bicucullin not shown) and the GABA_B receptor with GCP 55845 had no effect on GABA_A induced vasorelaxation at the highest concentrations of these compounds, whereas blocking of the GABA_C receptor with TPMPA totally blocked GABA induced vasorelaxation.

25 Detailed description of the invention

Definitions:

GABA-A, B and C receptors

30 The GABA-rho receptor (also known as the GABA_C receptor) is a subclass of GABA_A receptors composed of rho (ρ) subunits. The GABA_C receptors are chloride channels, and like other ligand-gated ion channels the five subunits are arranged around a symmetry axis to form a central ion conducting pore. Three different ρ subunit types have been identified denoted as ρ1, ρ2 and ρ3. The GABA_C receptor may either be a
35 homopentamer consisting of ρ1₅, ρ2₅ or ρ3₅ or a heteropentamer consisting of ρ2_m, ρ3_n

or $\rho 1_m$, $\rho 2_n$ (where $m+n = 5$). The GABA_A- ρ receptor, like other GABA_A receptors, is expressed in many areas of the brain, but has especially high expression in the retina.

Agonist

- 5 An agonist of the present invention may be a partial agonist, a full agonist or super-agonist defined with respect to the activity of GABA. A full receptor agonist is a compound that binds to a receptor of a cell and triggers a response by that cell. Agonists often mimic the action of a naturally occurring substance.
- 10 Partial agonists bind and activate a given receptor, but have only partial efficacy at the receptor relative to a full agonist.
- Superagonists are a type of agonist that is capable of producing a maximal response greater than the endogenous agonist for the target receptor, and thus has an efficacy of more than 100%.
- 15 An agonist of the present invention does not agonise GABA_A and/or GABA_B substantially.

Antagonist

- An antagonist of the present invention may be a partial antagonist or a full antagonist. A full receptor antagonist is a compound that does not provoke a biological response
- 20 itself upon binding to a receptor, but blocks or dampens agonist-mediated responses. In pharmacology, antagonists have affinity but no efficacy for their cognate receptors, and binding will disrupt the interaction and inhibit the function of an agonist or inverse agonist at receptors.
- Partial antagonists bind and dampen agonist-mediated responses, but have only partial
- 25 efficacy at the receptor relative to a full agonist.
- An antagonist of the present invention does not antagonise GABA_A and/or GABA_B substantially.

"Treating" refers to:

- 30 (i) preventing a disease, disorder or condition from occurring in an animal that may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it;
- (ii) inhibiting the disease, disorder or condition are ionotropic receptors, i.e., arresting its development; and/or they are ligand-gated ion channels.

(iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or condition.

"Treating a retinal disorder" refers to:

- 5 (i) preventing a retinal disorder from occurring in an animal that may be predisposed to a retinal disorder but has not yet been diagnosed as having it;
(ii) inhibiting or slowing a retinal disorder, e.g. arresting its development; and/or
(iii) relieving a retinal disorder, e.g. causing its regression.

10 **Vasoconstriction**

Vasoconstriction is the narrowing of the blood vessels resulting from contraction of the muscular wall of the vessels, particularly the large arteries, small arterioles and veins. The process is the opposite of vasodilation, the widening of blood vessels. The process is particularly important in staunching hemorrhage and acute blood loss. When blood
15 vessels constrict, the flow of blood is restricted or decreased, thus, retaining body heat or increasing vascular resistance. Cutaneously, this makes the skin turn paler because less blood reaches the surface, reducing the radiation of heat. On a larger level, vasoconstriction is one mechanism by which the body regulates and maintains mean arterial pressure.

20 Substances causing vasoconstriction are called vasoconstrictors or vasopressors. Generalized vasoconstriction usually results in an increase in systemic blood pressure, but it may also occur in specific tissues causing a localized reduction in blood flow. The extent of vasoconstriction may be slight or severe depending on the substance or circumstance.

25 In one embodiment the compound of the present invention is used to prevent or promote vasoconstriction. An agonist of the present invention may act to prevent vasoconstriction, while an agonist of the present invention may act to promote vasoconstriction.

Vasodilation

30 Vasodilation refers to the widening of blood vessels resulting from relaxation of smooth muscle cells within the vessel walls, particularly in the large arteries, smaller arterioles and large veins. The process is essentially the opposite of vasoconstriction, or the

narrowing of blood vessels. When vessels dilate, the flow of blood is increased due to a decrease in vascular resistance. Therefore, dilation of arterial blood vessels (mainly arterioles) leads to a decrease in blood pressure. The response may be intrinsic (due to local processes in the surrounding tissue) or extrinsic (due to hormones or the nervous system). Additionally, the response may either be localized to a specific organ (depending on the metabolic needs of a particular tissue, as during strenuous exercise), or systemic (seen throughout the entire systemic circulation). Factors that result in vasodilation are termed vasodilators.

In one embodiment the compound of the present invention is used to prevent or promote vasodilation. An agonist of the present invention may act to promote vasodilation, while an antagonist of the present invention may act to prevent vasodilation.

Vasospasms

Vasospasm refers to a condition in which blood vessels spasm, leading to vasoconstriction. This can lead to tissue ischemia and death (necrosis). Cerebral vasospasm may arise in the context of subarachnoid hemorrhage. Symptomatic vasospasm or delayed cerebral ischemia is a major contributor to post-operative stroke and death especially after aneurysmal subarachnoid hemorrhage. Vasospasm typically appears 4 to 10 days after subarachnoid hemorrhage.

In one embodiment the compound of the present invention is used to prevent or promote vasospasms. An agonist of the present invention may act to prevent vasospasms, while an antagonist of the present invention may act to promote vasospasms.

GABA receptors

γ -aminobutyric acid (GABA) is the main inhibitory transmitter in the nervous system. GABA acts via three distinct receptor classes: A, B and C.

The GABA_A and GABA_C receptors belong to the nicotinicoid superfamily and accordingly GABA_A and GABA_C receptors are ionotropic receptors, i.e. they are ligand-gated ion channels.

GABA_B receptors are metabotropic, i.e. they act via a G-protein-coupled second messenger system. They are slower than ionotropic receptors and usually modulate

the response of the neuron rather than participating in the task of the fast-acting ionotropic receptors.

GABA_C receptors have a distinct pharmacology to that of GABA_A and GABA_B receptors. GABA_C receptors are not inhibited by the alkaloid bicuculline or affected by benzodiazepines and barbiturates, which characteristically affect GABA_A receptors. Furthermore, GABA_C receptors are not activated by baclofen or inhibited by phaclofen, which typically act at GABA_B receptors (Chebib et al., J Pharmacol Exp Ther; 328:448-457; 2009).

The present invention relates to specific agonists and specific antagonist of the GABA_C receptor. Agonist and antagonist are defined herein above and below.

GABA_C receptor

GABA_C receptors are about 10-fold more sensitive to GABA than GABA_A receptors and the GABA_C receptors have a higher presence of ligand binding sites and more cooperative binding. The ion currents through GABA_C receptors are smaller and they activate and deactivate more slowly than GABA_A receptors. GABA_C receptors do not desensitize even with long agonist applications.

The GABA_C receptors are chloride channels formed from five rho (ρ) subunits. Like other ligand-gated ion channels the five subunits are arranged around a fivefold symmetry axis to form a central ion conducting pore. Three different ρ subunits have been identified termed as $\rho 1$, $\rho 2$ and $\rho 3$. The GABA_C receptor may be either a homopentamer consisting of $\rho 1_5$, $\rho 2_5$ or $\rho 3_5$ or the GABA_C receptor may be a heteropentamer consisting of $\rho 2_m$, $\rho 3_n$ or $\rho 1_m$, $\rho 2_n$ (where $m+n = 5$) (Enz, R. Vision Res 38(19): 1431-41, 1998).

The importance of the GABA_C receptors has evolved in recent years and they have been identified to be implicated in sleep-waking processes, memory enhancement, and modulation of eye growth and refractive development and it has been suggested that drugs acting on GABA_C receptors would make for potential treatments for sleep disturbances, cognitive disorders and myopia (Abdel-Halim, H. Chem Biol Drug Des; 71:306-327; 2008).

The GABA_C receptor has been found to be most extensively expressed in the retina and has been found in the retina of all vertebrate species investigated (Enz, R., Biol Chem, 382:1111-1122; 2001 and Lukasiewicz, Mol Neurobiol, 12(3):181-94.1996).

5 Using RT-PCR the GABA_C receptor has also been found in various parts of the vertebrate brain (thalamus, cerebellum, mesencephalon, temporal cortex, frontal cortex and occipital cortex), the spinal cord, the thymus, the pituitary gland, the heart, the liver, the adrenal gland, the gonadal endocrine tissues and placenta.

GABA_C receptor-ligands

10 GABA_C receptor-ligands range from agonists, antagonist and allosteric modulators. The pharmacological profile for this receptor differs from both GABA_A and GABA_B receptors.

A receptor agonist is a compound that binds to a receptor of a cell and triggers a response by that cell. Agonists often mimic the action of a naturally occurring
15 substance. A GABA_C specific agonist of the present invention has a lower EC₅₀ value for the GABA_C receptor than for GABA_A and/or GABA_B, such as the naturally occurring GABA receptor ligand GABA.

20 With regard to the present invention any naturally occurring ligand is not considered an agonist.

A receptor antagonist is a compound that does not provoke a biological response itself upon binding to a receptor, but blocks or dampens agonist-mediated responses. In pharmacology, antagonists have affinity but no efficacy for their cognate receptors, and
25 binding will disrupt the interaction and inhibit the function of an agonist or inverse agonist at receptors. A GABA_C specific antagonist of the present invention has a lower IC₅₀ value for the GABA_C receptor than for GABA_A and/or GABA_B.

Specific agonists

30 To date, the most selective agonist at GABA_C receptors is CACA, muscimol, (+)-ACPECA, (±)-CAMP, specifically (+)-(1R, 2S)-CAMP and 2-FTACA, (S)-2-MeGABA, TACA, (-)-TACP (Abdel-Halim et al., Chem Biol Drug Des, 71:306-327; 2008), 2-methyl-imidazole-acetic acid and anti-GABA-C activating antibodies.

In a preferred embodiment of the present invention the specific GABAC agonist is selected from the group consisting of: (+)-CAMP, (+)-(1R, 2S)-CAMP, 2-FTACA, TACA and 2-methyl-imidazole-acetic acid.

5 *Specific antagonists*

The most specific GABA_C receptor antagonist is TPMPA although it is also a weak antagonist of GABA_A receptors and a weak agonist of GABA_B receptors. Other selective antagonist of GABAC receptor is TPEPA, ACPMPA, cis-3-ACPMPA, trans-3-ACPMPA, ACPBuPA, cis-3-ACPBPA, trans-3-ACPBPA (Chebib et al. J Pharmacol Exp
10 Ther, 328:448-457; 2009; Abdel-Halim et al., Chem Biol Drug Des, 71:306-327; 2008), neutralizing antibodies and silencing RNA.

In a preferred embodiment of the present invention the specific GABA_C antagonist is selected from the group consisting of: TPEPA and TPMPA.

15 **Antibodies**

An antibody according to the invention is a polypeptide or protein capable of recognising and binding an antigen comprising at least one antigen binding site. The antibody may be directed against any one of the GABA_C subunits. Preferably the antibody is directed against an epitope conserved among the GABA_C subunits.

20

In a preferred embodiment said antigen is located in the extracellular domain of GABA_C receptor, more preferably in the GABA_C receptor binding site. The antibody may be a naturally occurring antibody, a fragment of a naturally occurring antibody or a synthetic antibody. Methods for generation, selection, cloning and expression of antibodies are
25 well known in the art.

25

The antibody according to the invention may be a monoclonal antibody, such as a naturally occurring monoclonal antibody or it may be polyclonal antibodies, such as naturally occurring polyclonal antibodies.

30

Interfering RNA

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA molecules, 20-25 nucleotides in length, that play a variety of roles in biology. The most notable role of siRNA is its involvement in

the RNA interference (RNAi) pathway, where it interferes with the expression of a specific gene.

siRNA according to the present invention is a short (20-25 nucleotides) strand of RNA, PNA or LNA nucleotide which is capable of hybridising with a GABA_C subunit transcript and thereby silence the expression of the receptor or receptor subunit. Preferably more than one siRNA is used to ensure that expression of all subunits is silenced by using siRNA molecules directed against all the different subunits.

Methods for designing, testing and manufacturing siRNA are well known in the art.

GABA_C assay

To evaluate a ligand (agonist or antagonist) and the specificity and efficiency a suitable assay is required.

Several assays for measuring the activity of ion channels exist.

Among the ligands that can be identified by the assay are natural ligand compounds of the ion channel; synthetic analogs and derivatives of natural ligands; antibodies, antibody fragments, and/or antibody-like compounds derived from natural antibodies, neutralising antibodies; and/or synthetic compounds identified by high-throughput screening of libraries; and the like. The assay may be performed using single putative ligands, and/or may be performed using a known ligand in combination with candidate ligands.

Patch clamping is considered the standard experiment for measuring membrane potential, but it is a very slow and labor-intensive method if you want to use it in a primary screen. One assay which may be used to identify ligands that modulate the activity of the GABA_C receptor is the FLIPR assay (Molecular Devices). Changes in plasma membrane potential correlate with the modulation of ion channels as ions move into or out of the cell. The FLIPR system measures such changes in membrane potential. This is accomplished by loading cells expressing an ion channel gene with a cell-membrane permeating fluorescent indicator dye suitable for measuring changes in membrane potential such as DiBAC (bis-(1,3-dibutylbarbituric acid) pentamethine oxonol, Molecular Probes). Thus the effect of a given ligand is evaluated based on the modulation of ion channel activity which is assessed with FLIPR and detected as changes in the emission spectrum of the DiBAC dye.

DiBAC is a lipophilic, anionic, bis-oxonol dye which can partition across the cytoplasmic membrane of live cells, dependent on the membrane potential across the plasma membrane. DiBAC is qualified as a “slow dye” because this dye partitioning is slow. The membrane potential assay utilizes one of DiBAC’s properties; its fluorescence intensity increases when the dye is bound to cytosolic proteins.

In this assay the cells are first washed with a balanced salt solution, so serum or other growth medium constituents are removed from the extracellular environment. DiBAC is subsequently added to the cells to equilibrate across the plasma membrane. During the dye loading/equilibration time, compounds are prepared in a DiBAC solution identical to the dye loading preparation. The microplate containing the cells is then transferred to the FLIPR instrument where compounds and controls are added to the cells while the fluorescence signal is monitored. When the cells are depolarized, more DiBAC enters the cells, and the increased concentration of DiBAC binding to intracellular lipids and proteins causes an increase in fluorescence signal. When the cells are hyperpolarized, DiBAC exits the cells and the decreased concentration of DiBAC binding to lipids and proteins results in a decrease of fluorescence signal. Throughout the assay, the temperature is maintained constant at 36°C since DiBAC’s extinction coefficient is very temperature sensitive. DiBAC excites at 488 nm and emits in the 510 nm-570 nm range.

The FLIPR assay may be used both with adherent and non-adherent cells.

The effect of a given compound as measured in the FLIPR assay may be compared to the effect of a known agonist and/or antagonist of the GABA_C receptor. Dose-response curves for agonists and antagonists in the FLIPR assay may be constructed on the basis of the maximal responses at different concentrations of the respective ligands.

In another embodiment the effect of GABA_C specific agonists and antagonists are tested *in vitro* on retinal arterioles freshly removed from an eye (the “arteriole diameter” assay). The arterioles are mounted in a pipette system for isobaric studies or in a myograph for isometric studies. The compound is added in increasing concentrations and the diameter or tone produced in the vessel after this addition of compound is recorded.

The effect of GABA_C specific agonists and antagonists are tested *in vivo* by photographing the retina through the optics of the eye and measuring the changes in diameter of the vessels on these photographs after addition of the compound.

5 The effect of a given compound as measured in the “arteriole diameter” assay may be compared to the effect of a known agonist and/or antagonist of the GABA_C receptor. Dose-response curves for agonists and antagonists in the “arteriole diameter” assay may be constructed on the basis of the maximal responses at different concentrations of the respective ligands.

10

For functional antagonist assays the most common summary measure of the dose-response curve is the IC₅₀; the concentration of substance that provides 50% inhibition. For agonist assays the most common summary measure is the EC₅₀; the concentration giving 50% of that compound's maximal response.

15

Once these dose-response measurements have been performed it is possible to calculate the IC₅₀/EC₅₀ value of a given compound. Based on this value it may be evaluated whether the given compound is a specific agonist or antagonist by comparing the EC₅₀ value obtained for the ligand when interacting with the GABA_A,
20 GABA_B or GABA_C receptor.

20

According to this invention a GABA_C specific agonist has an EC₅₀ value in the range of 0.1 μM to 1.0 mM. This EC₅₀ value should also be significantly ($p < 0.05$) different from the EC₅₀ value obtained when the compound interacts with either the GABA_A or GABA_B
25 receptor. Preferably the EC₅₀ value for the interaction with the GABA_C receptor is reduced by at least a factor 2 when compared to the EC₅₀ value for the interaction of the same compound to the GABA_A or GABA_B receptor, more preferably the EC₅₀ value is reduced by at least a factor 2.5, such as a factor 3, more preferably by at least a factor 3.5, such as a factor 4, more preferably by at least a factor 4.5, such as a factor
30 5, more preferably by at least a factor 5.5, such as a factor 6, more preferably at least a factor 7, such as a factor 7.5, more preferably at least a factor 10, such as a factor 15, more preferably at least a factor 20, such as a factor 25, more preferably at least a factor 30, such as a factor 35, more preferably at least a factor 40, such as a factor 45, more preferably at least a factor 50, such as a factor 55, more preferably at least a
35 factor 60, such as a factor 65, more preferably at least a factor 70, such as a factor 75,

more preferably at least a factor 80, such as a factor 85, more preferably at least a factor 90, such as a factor 95, most preferably by more than a factor 100.

5 According to this invention a GABA_C specific antagonist has an IC₅₀ value in the range of 1 μ M to 1 mM. The IC₅₀ value should also be significantly ($p < 0.05$) different from the IC₅₀ value obtained when the compound interacts with either the GABA_A or GABA_B receptor.

10 Preferably the IC₅₀ value for the interaction with the GABA_C receptor is reduced by at least a factor 2 when compared to the IC₅₀ value for the interaction of the same compound to the GABA_A or GABA_B receptor, more preferably the IC₅₀ value is reduced by at least a factor 2.5, such as a factor 3, more preferably by at least a factor 3.5, such as a factor 4, more preferably by at least a factor 4.5, such as a factor 5, more preferably by at least a factor 5.5, such as a factor 6, more preferably at least a factor 7, such as a factor 7.5, more preferably by at least a factor 10, such as a factor 15, 15 more preferably at least a factor 20, such as a factor 25, more preferably at least a factor 30, such as a factor 35, more preferably at least a factor 40, such as a factor 45, more preferably at least a factor 50, such as a factor 55, more preferably at least a factor 60, such as a factor 65, more preferably at least a factor 70, such as a factor 75, more preferably at least a factor 80, such as a factor 85, more preferably at least a factor 90, such as a factor 95, most preferably by more than a factor 100. 20

Dosages

The dosage requirements will vary with the particular drug composition employed, the route of administration and the particular subject being treated. It will also be 25 recognized by one of skill in the art that the optimal quantity and spacing of individual dosages of a compound or a pharmaceutically acceptable salt thereof will be determined by the nature and extent of the condition being treated, the form, route and site of administration, and the particular patient being treated, and that such optimums can be determined by conventional techniques. It will also be appreciated by one of 30 skill in the art that the optimal course of treatment, i.e., the number of doses of a compound or a pharmaceutically acceptable salt thereof given per day for a defined number of days, can be ascertained using conventional course of treatment determination tests.

In the context of the present invention, the term “daily dosage” is meant to describe the daily dosage required for an average human subject having a weight of about 70 kg. In general, for administration to human patients the daily dosage level of the compounds in accordance with the present invention, is in a range of from about 0.1 mg to about 100 mg.

In one embodiment of the invention the compound is given in a daily dosage in a range of from about 0.1 mg to about 50 mg, such as e.g., from about 0.2 mg to about 50 mg, about 0.3 mg to about 50 mg, about 0.4 mg to about 50 mg, about 0.5 mg to about 50 mg, about 0.6 mg to about 50 mg, about 0.7 mg to about 50 mg, about 0.8 mg to about 50 mg, about 0.9 mg to about 50 mg, about 1 mg to about 50 mg, about 1.5 mg to about 50 mg, about 1.75 mg to about 50 mg, about 2.0 mg to about 50 mg, about 2.5 mg to about 50 mg, about 3.0 mg to about 50 mg, about 3.5 mg to about 50 mg, about 4.0 mg to about 50 mg, about 4.5 mg to about 50 mg, about 5.0 mg to about 50 mg, about 5.5 mg to about 50 mg, about 6.0 mg to about 50 mg, about 6.5 mg to about 50 mg, about 7.0 mg to about 50 mg, about 7.5 mg to about 50 mg, about 8.0 mg to about 50 mg, about 8.5 mg to about 50 mg, about 9.0 mg to about 50 mg, about 9.5 mg to about 50 mg, about 10 mg to about 50 mg, about 20 mg to about 50 mg, about 30 mg to about 50 mg, or about 40 mg to about 50 mg.

In another embodiment of the invention the compound is given in a daily dosage in a range of from about 50 mg to about 100 mg, such as e.g., from about 55 mg to about 100 mg, about 65 mg to about 100 mg, about 75 mg to about 100 mg, about 80 mg to about 100 mg, about 85 mg to about 100 mg, about 90 mg to about 100 mg, about 95 mg to about 100 mg, or about 98 mg to about 100 mg.

In a preferred embodiment of the invention the compound is 2-methyl-imidazole acetic acid and the daily dosage is in a range of from about 0.1 – 100 mg.

In another embodiment the administration is given directly into the eye of the subject to be treated. The term “daily dosage” will then describe the daily dosage administered per eye. The dosage administered directly into the eye can vary quite a lot without any side effects because of the blood-retinal barrier. In general, for administration to human patients directly to the eye using eye drops, the daily dosage level of the compounds in accordance with the present invention is in a range of from 0.1 mg/ml to about 20

mg/ml. The eye drops according to the present invention may be administered 1 to 10 times daily, more preferably 1-6 times per day, such as 1-4, or 1-2 times per day. On each occasion it is normally sufficient to administer 1 drop per eye because the volume of a drop exceeds the required volume.

5

The drop is a unit of measure of volume, the amount dispensed as one drop from a dropper. It is often used in giving quantities of liquid drugs to patients. A medical drop equals 1/12 mL (83⅓ μL).

10 Eye drops may be administered as liquid eyedrops or as an ointment.

The skilled person will readily be able to determine the dosage levels required for a subject whose weight falls outside the average range, such as children and the elderly. The daily dosage may optionally be administered as a single dose or be divided in two or more doses, such as e.g. two, three, or four, for administration at different times during the day. The skilled person will appreciate that, in the regulation of blood flow or diseases associated with regulation of blood flow, a compound used in accordance with the presents invention may be taken as a single dose on an "as required" basis, i.e., as needed. The physician will in any event determine the actual dosage which will be most suitable for any particular patient and it will vary with the age, weight and response of the particular patient. The above dosages are, of course only exemplary of the average case and there may be instances where higher or lower doses are merited and such are within the scope of the invention.

25 Another way of expressing the daily dosage level in accordance with the present invention is as mg/kg. Accordingly, for administration to human patients the daily dosage levels of the compounds in accordance with the present invention, or pharmaceutically acceptable salts, solvates or prodrugs thereof, will be in a range from about 0.0015 mg/kg to about 1.5 mg/kg, preferably from about 0.0050 mg/kg to about 1.5 mg/kg, more preferably from about 0.01 mg/kg to about 1.5 mg/kg, more preferably from about 0.05 mg/kg to about 1.5 mg/kg, more preferably from about 0.1 mg/kg to about 1.5 mg/kg, more preferably from about 0.2 mg/kg to about 1.5 mg/kg, more preferably from about 0.4 mg/kg to about 1.5 mg/kg, more preferably from about 0.6 mg/kg to about 1.5 mg/kg, more preferably from about 0.8 mg/kg to about 1.5 mg/kg, more preferably from about 1.0 mg/kg to about 1.5 mg/kg, more preferably from about

1.1 mg/kg to about 1.5 mg/kg, more preferably from about 1.2 mg/kg to about 1.5 mg/kg, more preferably from about 1.3 mg/kg to about 1.5 mg/kg, more preferably from about 1.4 mg/kg to about 1.5 mg/kg.

5 **Pharmaceutical formulations**

Whilst it is possible for the compounds or salts of the present invention to be administered as the raw chemical, it is preferred to present them in the form of a pharmaceutical formulation. Accordingly, the present invention further provides a pharmaceutical formulation, which comprises a compound of the present invention or a pharmaceutically acceptable salt or ester thereof, as herein defined, and a pharmaceutically acceptable carrier therefore. The pharmaceutical formulations may be prepared by conventional techniques, e.g. as described in Remington: The Science and Practice of Pharmacy 2005, Lippincott, Williams & Wilkins.

The pharmaceutically acceptable carriers can be either solid or liquid. Solid form preparations include powders, tablets, pills, capsules, cachets, suppositories, and dispersible granules. A solid carrier can be one or more excipients which may also act as diluents, flavoring agents, solubilizers, lubricants, suspending agents, binders, preservatives, wetting agents, tablet disintegrating agents, or an encapsulating material.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for oral administration. Such liquid forms include solutions, suspensions, and emulsions. These preparations may contain, in addition to the active component, colorants, flavors, stabilizers, buffers, artificial and natural sweeteners, dispersants, thickeners, solubilizing agents, and the like.

The compounds of the present invention may be formulated for parenteral administration and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers, optionally with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, for example solutions in aqueous polyethylene glycol. Examples of oily or non-aqueous carriers, diluents, solvents or vehicles include propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters (e.g., ethyl oleate), and may contain agents such as preserving, wetting,

emulsifying or suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution for constitution before use with a suitable vehicle, e.g., sterile, pyrogen-free water.

5

The compounds of the invention may also be formulated for topical delivery. The topical formulation may include a pharmaceutically acceptable carrier adapted for topical administration. Thus, the composition may take the form of a suspension, solution, ointment, lotion, sexual lubricant, cream, foam, aerosol, spray, suppository, implant, inhalant, tablet, capsule, dry powder, syrup, balm or lozenge, for example.

10

Preferably, the formulation will comprise about 0.5% to 75% by weight of the active ingredient(s) with the remainder consisting of suitable pharmaceutical excipients as described herein.

15

Pharmaceutically acceptable salts of the instant compounds, where they can be prepared, are also intended to be covered by this invention. These salts will be ones which are acceptable in their application to a pharmaceutical use. By that it is meant that the salt will retain the biological activity of the parent compound and the salt will not have untoward or deleterious effects in its application and use in treating diseases.

20

Pharmaceutically acceptable salts are prepared in a standard manner. If the parent compound is a base it is treated with an excess of an organic or inorganic acid in a suitable solvent. If the parent compound is an acid, it is treated with an inorganic or organic base in a suitable solvent.

25

The compounds of the invention may be administered in the form of an alkali metal or earth alkali metal salt thereof, concurrently, simultaneously, or together with a pharmaceutically acceptable carrier or diluent, especially and preferably in the form of a pharmaceutical composition thereof, whether by oral, rectal, or parenteral (including subcutaneous) route, in an effective amount.

30

Examples of pharmaceutically acceptable acid addition salts for use in the present inventive pharmaceutical composition include those derived from mineral acids, such as hydrochloric, hydrobromic, phosphoric, metaphosphoric, nitric and sulfuric acids,

35

and organic acids, such as tartaric, acetic, citric, malic, lactic, fumaric, benzoic, glycolic, gluconic, succinic, p-toluenesulphonic acids, and arylsulphonic, for example.

5 **Routes of administration**

Systemic treatment

The main routes of administration are oral and parenteral in order to introduce the compound into the blood stream to ultimately target the sites of desired action.

10 Appropriate dosage forms for such administration may be prepared by conventional techniques.

Oral administration

15 Oral administration is normally for enteral drug delivery, wherein the compound is delivered through the enteral mucosa.

Parenteral administration

Parenteral administration is any administration route not being the oral/enteral route whereby the medicament avoids first-pass degradation in the liver. Accordingly, 20 parenteral administration includes any injections and infusions, for example bolus injection or continuous infusion, such as intravenous administration, intramuscular administration, subcutaneous administration. Furthermore, parenteral administration includes inhalations and topical administration.

25 Accordingly, the compound may be administered topically to cross any mucosal membrane of an animal to which the biologically active substance is to be given, e.g. in the nose, vagina, eye, mouth, genital tract, lungs, gastrointestinal tract, or rectum, preferably the mucosa of the eye, and accordingly, parenteral administration may also include buccal, sublingual, nasal, rectal, vaginal and intraperitoneal administration as 30 well as pulmonal and bronchial administration by inhalation or installation. Also, the compound may be administered topically to cross the skin.

The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

35

Local treatment

The compound according to the invention may be used as a local treatment, ie. be introduced directly to the site(s) of action.

Accordingly, the compound may be applied topically or injected into the site of action, for example by injection into the aqueous humour of eye.

5

Ophthalmic disorders

The GABA_C agonist and/or antagonists of the present invention may be used to treat ophthalmic disorders.

10 Ophthalmic disorders of the present invention may be selected from the group below: retinitis pigmentosa, macular degeneration, cone-rod dystrophy, retinal separation, hypertensive retinopathy, diabetic retinopathy, retinal dysplasia, progressive retinal atrophy, retinal degeneration, retinal vascular occlusion, radiation retinopathy, sickle cell retinopathy, Coat's disease, and retinopathy of prematurity.

15

In one embodiment of the present invention the compound of the invention is administered locally to the eye to regulate blood flow in the retina.

***In vivo* models of Retinopathies**

20 In several *in vivo* and *in vitro* models of retinopathies with different underlying causes of retinal neuronal death effects of compounds have been described. These *in vitro* and *in vivo* models may be used to verify the utility of agonists and antagonists of the present invention in the treatment of retinopathies.

25 In humans, mutations in several genes have been shown to cause photoreceptor degeneration resulting in visual loss and blindness. Examples include genes encoding the rod β -subunit of the cyclic GMP phosphodiesterase, rhodopsin and peripherin. Transgenic mice carrying such mutations have been generated and may be used to test compounds *in vivo* for effect in photoreceptor diseases including retinitis
30 pigmentosa and macular degeneration (Portera-Cailliau et al., 1994 PNAS 91, 974-978).

The rd1 retinal degeneration mouse, exhibits a rapid retinal degeneration initiated by a recessive mutation of the gene encoding the rod 1-subunit of the cyclic GMP
35 phosphodiesterase. The phenotype and genetic mutation correspond well to the some

of forms of Retinitis Pigmentosa (RP) and this mouse model has been widely studied for more than 70 years.

5 The rd1 mice develop fully differentiated photoreceptors during the second postnatal week, but then suffer nearly complete loss of the rod photoreceptors in the next week, followed by a slower loss of cone photoreceptors (Carter-Dawson et al., 1978 IOVS 17, 489-498).

10 Protective effects of various compounds on the photoreceptors have been described in various experimental set-ups using the rd1 mouse. Subretinal injections of GDNF protein into P13-P17 rd1 mice resulted in both histological and functional neuroprotection of the rod photoreceptors (Frasson et al., 1999 IOVS 40, 2724-2734). In organ cultures using retinal explants from neonatal rd1 retina, addition of lens epithelium-derived growth factor (LEDGF), BDNF, and CNTF rescued photoreceptors
15 to different extent (Caffe et al., 2001 IOVS 42, 275-282; Ahuja et al 2001 Neuroreport 12, 2951-2955).

Another widely used transgenic model to study photoreceptor degeneration is the Royal College of Surgeons (RCS) rat which carries a mutation in the MERTK gene that
20 renders RPE cells unable to phagocytose shed photoreceptor outer segments at a normal rate. This ultimately leads to photoreceptor death and visual dysfunction. Using topical delivery of CNTF to the retina, the degenerating photoreceptors in the RCS rat may be rescued (Huang et al., 2004 J Biomed Sci. 11, 37-48).

25 Intravitreal injections of AAV expressing GDNF have been shown to protect rat retina from ischemia-reperfusion injury by moderate preservation of the inner retina (Wu et al. 2004 Molecular Vision 10, 93-102). Although poorly understood, glutamate toxicity is thought to play major role in retinal ischemia following acute vascular occlusion.

30 Retinal detachment (RD) is a common cause of visual impairment that results in loss of photoreceptors. In an *in vivo* model, delivery of GDNF using an AAV vector has been shown to protect against RD-induced photoreceptor damage (Wu et al., 2002 IOVS 43, 3480-3488).

Other diseases

In one embodiment the compounds of the present invention may also be used in treatment of diseases related to an alternation of the blood flow, i.e. the compounds may be used as vasoconstrictor or vasodilator.

5

The GABA_C receptor has been located to various parts of the vertebrate brain (thalamus, cerebellum, mesencephalon, temporal cortex, frontal cortex and occipital cortex), the spinal cord, the thymus, the pituitary gland, the heart, the liver, the adrenal gland, the gonadal endocrine tissues and placenta, and accordingly the compounds of the present invention may be used in treatment of disease related to an altered blood flow in said organs.

10

In another embodiment of the present invention the compounds of the invention is used in treatment of thrombus formation.

15

Examples

The relaxing effect of GABA on retinal arterioles is mediated through the GABA_C receptor.

Solutions and compounds

20

Solutions: Physiological saline solution (PSS) containing (in mM): NaCl 119, KCl 4.7, MgSO₄ 1.17, NaHCO₃ 25, KH₂PO₄ 1.18, CaCl₂ 1.6, EDTA 0.026, Glucose 5.5 and HEPES 5.0 (pH=7.4) was used for transportation, storage and for the pharmacological experiments. During dissection and normalization of the vessel diameter, a similar solution was used in which CaCl₂ had been omitted (Ca²⁺-free PSS).

25

*Compounds:***Tone modulators:**

Compounds were purchased from Sigma (Sigma-Aldrich, Vallensbaek, Denmark): The glutamate NMDA-receptor antagonist DL-2-amino-5-phosphonopentanoic acid (DL-APV) was used as a precondition for inducing the vasodilating effect of GABA. Subsequent pre-constriction was performed with the tromboxane analogue U46619 (9,11-dideoxy-11 α ,9 α -epoxymethano-prostaglandin F_{2 α})

30

GABA-mediators:

All compounds were purchased from Tocris Bioscience (Bristol UK), apart from TPMPA, isoguvacine and GABA which were purchased from Sigma (Sigma-Aldrich,

35

Vallensbaek, Denmark), and 2-methyl-imidazole acetic acid (Madsen et al, J Med Chem, 2007, structure 8a) generously provided by Bente Frølund, Faculty of Pharmaceutical Sciences, University of Copenhagen. Numbers in brackets refer to the respective product lists.

5

GABA-antagonists:

The GABA_A-antagonists bicucullin 10^{-6} M (0130) and picrotoxin 15×10^{-6} M (1128), the GABA_B-antagonist GCP 55845 5×10^{-6} M (1248), and the GABA_C-antagonist (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) 10×10^{-6} M (1040)

10

GABA-agonists:

GABA (0344), the GABA_A-agonist Isoguvacin, the GABA_B-agonist Baclofen (0796) and the GABA_C agonist, 2-methyl-imidazole acetic acid.

15 **Preparation of compounds:**

U46619, NMDA, and ATP were dissolved in distilled water as stock solutions 10^3 times the highest concentration used in the experiments and stored frozen for later use. All other compounds were prepared on the day of the experiment and dissolved in PSS.

20 Tissue:

Porcine eyes were collected from a local slaughter house and were transported to the laboratory in 4°C PSS within one hour. The eyes were bisected by a frontal section through the equator, the vitreous was removed, and the retina was detached from the underlying pigment epithelium by injection of PSS between these two structures.

25 Subsequently, an arteriolar segment with a length of < 2 mm with approximately 2 mm retinal tissue attached on each side of the vessel was dissected from the retina.

Mounting procedure

The vascular segment was placed in the chamber of a small vessel myograph (610M Multi-Myograph, Danish Myo Technology, Aarhus, Denmark) and mounted on 25 µm diameter tungsten wires. After mounting, the preparation was suspended freely in PSS between the myograph jaws, and bubbling of the bath was commenced with a mixture of 95% atmospheric air and 5% CO₂ to result in a pH of 7.4.

35 Normalization

Normalization was performed by increasing the arteriolar diameter in four steps in Ca^{2+} -free PSS solution, and measuring the passive tensions (corresponding to transmural pressures between 0 and approximately 70 mm Hg). This diameter-tension relationship was exponential, and the intercept between this curve and a straight line based on the Laplace equation (wall tension = transmural pressure x radius) with the transmural pressure set to 70 mmHg was calculated. Using the built-in micrometer screw, the jaws of the myograph were adjusted to 93.5% of the intercept length, at which the arteriole can develop the maximum tone (i.e. the optimal length for contraction of the vascular smooth muscle cells).

Experimental protocols:

Only one arteriole was used from each animal. For each experimental condition at least six observations were obtained. The glutamate NMDA receptor antagonist DL-APV was added in a concentration of 50×10^{-6} M in all fluids during the experiments which in preliminary experiments had been shown to be a precondition for obtaining GABA induced vasorelaxation.

PROTOCOL 1:

A) Equilibration:

The arterioles were allowed to equilibrate for a period of 10 min in PSS, in order for the tone to stabilize.

B) Pre-contraction:

The vessel was pre-contracted using 10^{-6} M U46619.

C) Concentrations-response experiments:

One of the following compounds were added to the myograph chamber in seven steps with one log unit interval from 10^{-9} to 10^{-3} M: GABA, the GABA_A-agonist Isoguvacin, the GABA_B-agonist Baclofen and the GABA_C agonist, 2-methyl-imidazole acetic acid.

D) Removal of the perivascular tissue:

The myograph was moved to a stereo microscope and the perivascular retinal tissue was gently removed within two minutes using two pairs of fine forceps (Inox 5) without touching the arterioles.

E) Repetition of the concentration-response experiment:

The myograph was replaced in the recording unit and the procedures described in steps A-C were repeated on the isolated arteriole.

5 F) Maximal relaxation

Finally, papaverine 10^{-4} M was added in order to determine maximal relaxation.

PROTOCOL 2:

The procedures from protocol 1 were repeated with the following modifications:

10 1) In four experimental series, the following was added to the tissue during the equilibrium phase to be present throughout the experiment: 1) The GABA_A-antagonists bicucullin 10^{-6} M and 2) picrotoxin 15×10^{-6} M, 3) The GABA_B-antagonist GCP 55845 5×10^{-6} M, and 4) The GABA_C-antagonist (1,2,5,6-tetrahydropyridin-4-yl)methylphosphinic acid (TPMPA) 10×10^{-6} M.

15

Data analysis

The tensions produced by the mounted arterioles were sampled at 1 Hz and displayed on a computer monitor as a function of time during the experiments. The data were stored in an Excel ® file for the subsequent analysis. The tone obtained after addition
20 of each concentration of an agonist was normalized to the tone produced after addition of 10^{-6} M U46619. The normalized tone was plotted as a function of the agonist concentration.

Statistical analysis

25 In each concentration-response experiment Repeated Measurements ANOVA was used to test whether the tone changed significantly after addition of increasing concentrations of agonist.

Student's paired t-test was used to test for differences in the tone response of
30 arterioles with and without perivascular retinal tissue for each concentration of GABA and GABA agonists.

Results:

Figure 1 shows the concentration dependent relaxation of retinal arterioles induced by GABA in the presence of perivascular retinal tissue and DL-APV in a concentration of 50 microM.

5 Figure 2 shows that none of the experiments showed vasodilation after addition of GABA or one of the GABA agonists to isolated retinal arterioles (dotted lines).

The left part (Figure 2A) of the figure shows that in the presence of the perivascular retinal tissue the GABA_A agonist Isoguvacine and the GABA_B agonist Baclofen had no effect on vascular tone, whereas the GABA_C agonist 5-methyl-imidazol-4-acetate
10 induced a significant relaxation of the retinal arterioles at the highest three concentrations.

The right part (Figure 2B) of the figure shows that in the presence of perivascular retinal tissue a blocking of the GABA_A receptor with picrotoxin (and bicucullin not
15 shown) and the GABA_B receptor with GCP 55845 had no effect on GABA induced vasorelaxation at the highest concentrations of these compounds, whereas blocking of the GABA_C receptor while TPMPA totally blocked GABA induced vasorelaxation.

Claims

1. A GABA_C specific agonist or antagonist for use in regulation of vascular blood flow.
2. The use of claim 1, wherein said agonist is used as a vasodilator.
3. The use of any of claim 2, in the treatment of vasospasms, including but not limited to Reynauld's syndrome and Reynauld's phenomenon, Coronary artery vasospasm, and Reversible cerebral vasoconstriction syndrome and migraine.
4. The use of claim 1, wherein said antagonist is used as a vasoconstrictor.
5. The use of any of the preceding claims, in the treatment of a disorder of the retina, brain, spinal cord, thymus, pituitary gland, heart, liver, adrenal gland, gonadal endocrine tissues and placenta.
6. The use of claim 5, wherein the brain disorder is selected from the group consisting of migraine, epilepsy, Alzheimer's disease, Parkinson's disease,
7. The use of claim 5, wherein the disorder involves reduced blood supply.
8. The use of claim 7, wherein the reduced blood supply leads to hypoxia.
9. The use of claim 5, wherein the disorder involves excessive blood supply.
10. The use of claim 9, wherein the excessive blood supply leads to hemorrhaging.
11. The use of any of the preceding claims 7 or 9, wherein the retinal disorder is selected from the group consisting of: retinitis pigmentosa, macular degeneration, cone-rod dystrophy, retinal separation, hypertensive retinopathy, diabetic retinopathy, retinal dysplasia, progressive retinal atrophy, retinal degeneration, retinal vascular occlusion, radiation retinopathy, sickle cell retinopathy, Coat's syndrome, retinopathy of prematurity.
12. The use of claim 11, wherein said retinal disorder is diabetic retinopathy.

13. The use of any of the preceding claims, wherein the GABA_C agonist substantially does not agonise GABA_A.
14. The use of any of the preceding claims, wherein the GABA_C agonist substantially does not agonise GABA_B.
15. The use of any of the preceding claims, wherein the GABA_C antagonist substantially does not antagonise GABA_A.
16. The use of any of the preceding claims, wherein the GABA_C antagonist substantially does not antagonise GABA_A.
17. The use of any of the preceding claims, wherein the GABA_C agonist is selected from the group consisting of: (+)-ACPECA, muscimol, (+/-)-cis-2-aminomethylcyclopropane ((±)-CAMP), (+)-(1R, 2S)-CAMP and 2-FTACA, (S)-2-MeGABA, trans-4-Aminocrotonic acid (TACA), cis-4-aminocrotonic acid (CACA), (-)-TACP and an activating anti-GABA-C receptor antibody.
18. The use of any of the preceding claims, wherein the GABA_C agonist is selected from the group consisting of (±)-CAMP, and (+)-(1R, 2S)-CAMP.
19. The use of any of the preceding claims, wherein the GABA_C agonist is a partial agonist, a full agonist or a superagonist.
20. The use of any of the preceding claims, wherein the GABA_C agonist has an EC₅₀ of less than 1 mM.
21. The use of any of the preceding claims, wherein the GABA_C antagonist is a partial antagonist, or a full antagonist.
22. The use of any of the preceding claims, wherein the GABA_C antagonist has an IC₅₀ of less than 1 mM.
23. The use of any of the preceding claims, wherein the GABA_C antagonist is selected from the group consisting of: anti-GABA_C receptor antibodies and a GABA_C transcript specific siRNA.

24. The use of any of the preceding claims, in the treatment of a mammal, including but not limited to human beings, dogs, cats, and horses.
25. The use of claim 24, in the treatment of a human being.
26. The use of any of the preceding claims, wherein said agonist is administered at a dosage of 0.0015 – 1.5 mg/kg body weight.
27. The use of any of the preceding claims, wherein said antagonist is administered at a dosage of 0.0015 – 1.5 mg/kg body weight.
28. The use of any of the preceding claims, wherein said agonist or antagonist is administered systemically.
29. The use of any of the preceding claims, wherein said agonist or antagonist is administered locally.
30. The use of claim 29, wherein said local administration is topical administration to the eye, or injection into the aqueous humour of the eye.
31. The use of any of the preceding claims, wherein the vascular blood flow is regulated in at least one artery, at least one arteriole or at least one vein.
32. The use of claim 31, wherein the vascular blood flow is regulated in at least one arteriole, preferably wherein the arteriole is a retinal arteriole.
33. A method of regulating vascular blood flow in a subject, said method comprising administering to a subject in need thereof a therapeutically efficient amount of a GABA_C specific agonist or a GABA_C specific antagonist.
34. Use of a GABA_C specific agonist or antagonist for the preparation of a medicament for regulation of vascular blood flow.
35. The use of claim 34, wherein the agonist or antagonist are as defined in any of the preceding claims.
36. The use of claim 34 or 35, wherein the regulation of vascular blood flow is as defined in any of the preceding claims.

Figure 1

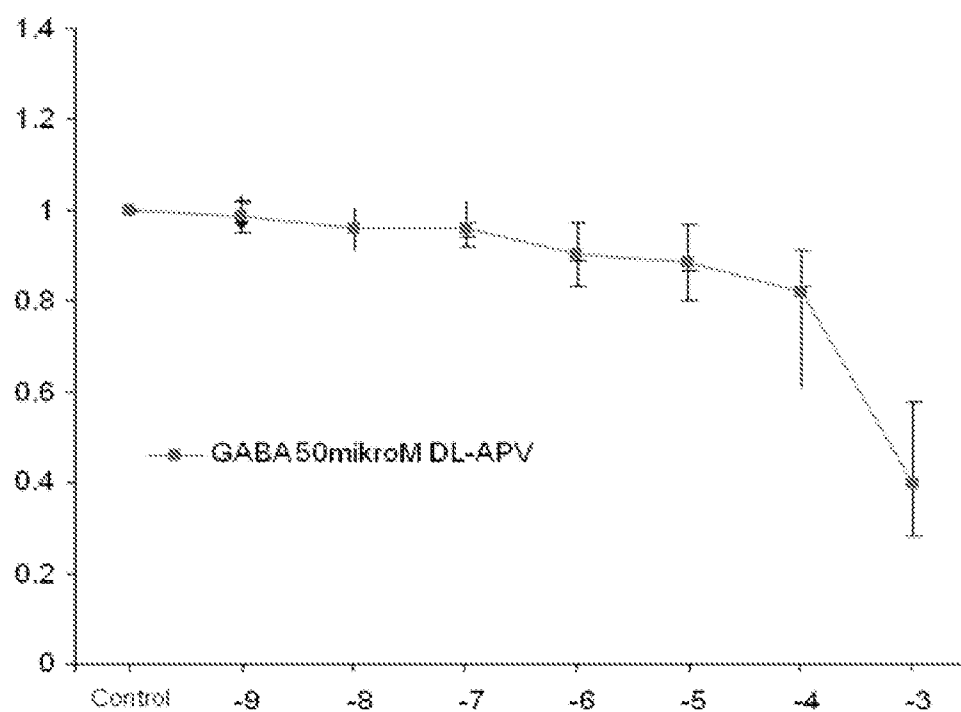


Figure 2A

Agonist experiments

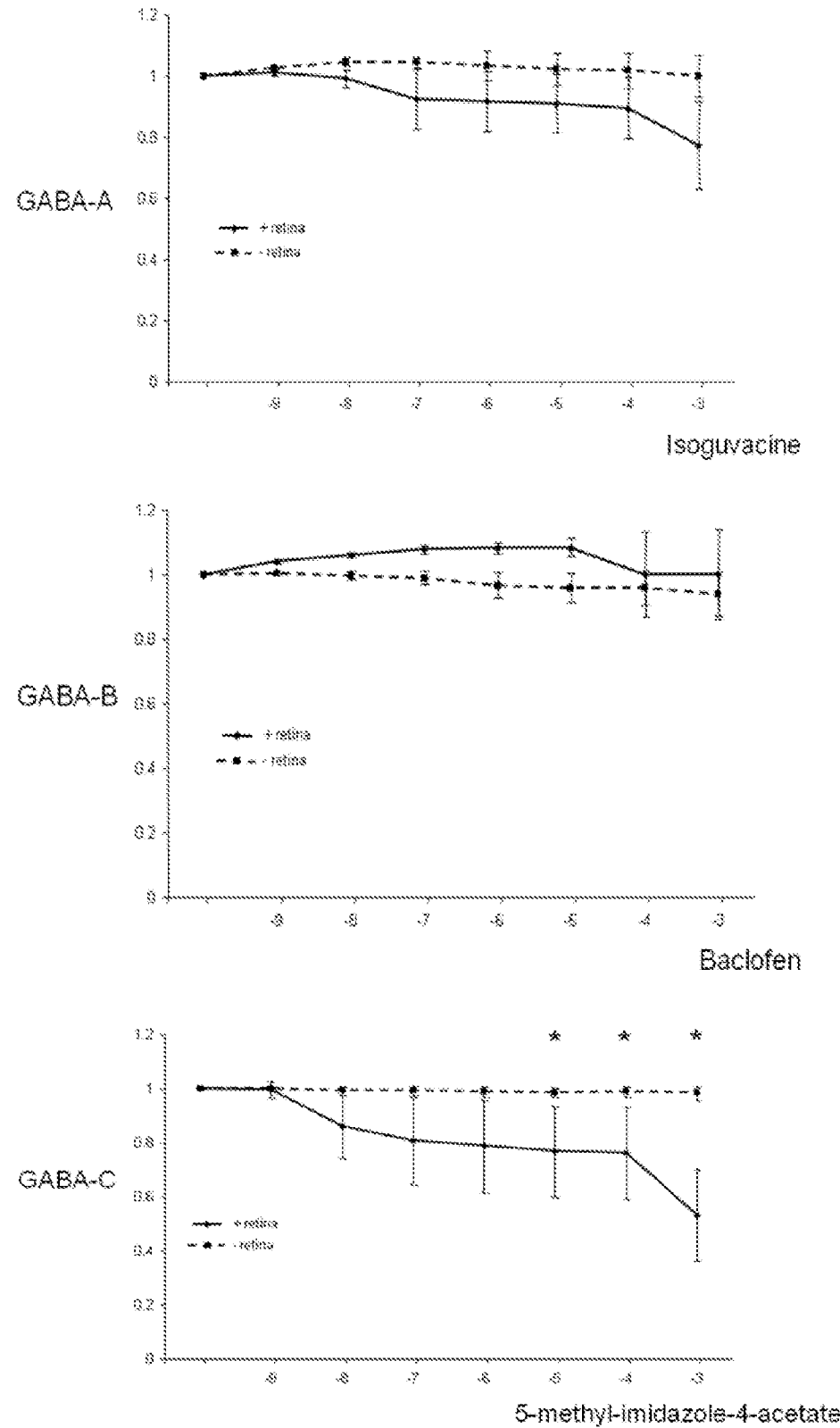
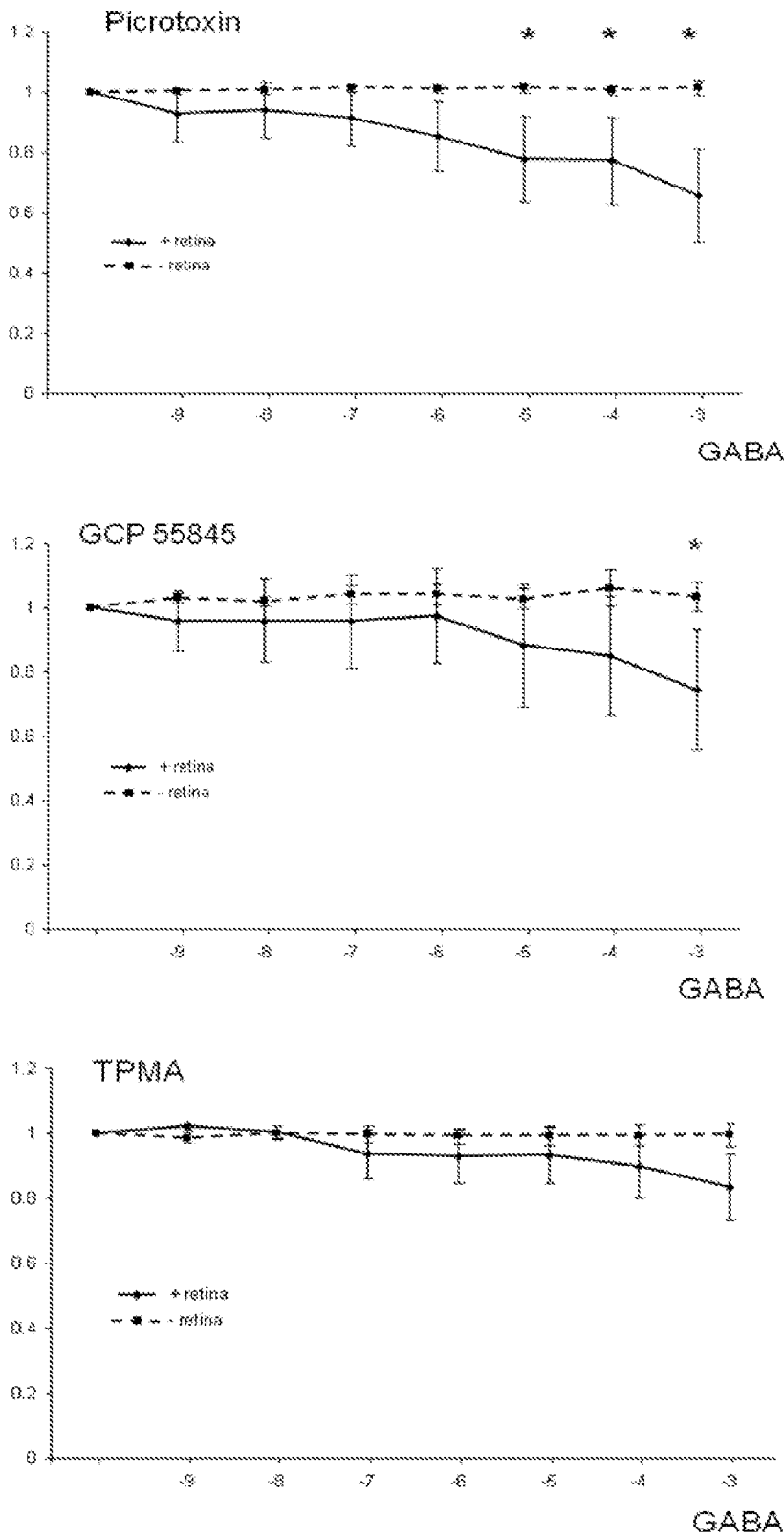


Figure 2B

Antagonist experiments



INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050077

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K45/06 A61K31/197 A61K31/4172 A61K31/42 A61P9/00
A61P25/00 A61P27/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data, EMBASE, BIOSIS, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ABDEL-HALIM HEBA ET AL: "A molecular basis for agonist and antagonist actions at GABA(C) receptors", CHEMICAL BIOLOGY & DRUG DESIGN (ONLINE), WILEY-BLACKWELL MUNKSGAARD, MUNKSGAARD DENMARK, UNITED KINGDOM, UNITED STATES, NETHERLANDS, vol. 71, no. 4, 1 April 2008 (2008-04-01), pages 306-327, XP009142090, ISSN: 1747-0285, DOI: 10.1111/J.1747-0285.2008.00642.X [retrieved on 2008-02-28] cited in the application page 306, column 2, paragraph 3 page 307, column 1, paragraph 2-3 table 1 ----- -/-	1-36

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

31 May 2012

Date of mailing of the international search report

14/06/2012

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INTERNATIONAL SEARCH REPORT

International application No
PCT/DK2012/050077

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOHNSTON GRAHAM A R ET AL: "Neurochemicals for the investigation of GABA(C) receptors.", NEUROCHEMICAL RESEARCH DEC 2010 LNKD- PUBMED:20963487, vol. 35, no. 12, December 2010 (2010-12), pages 1970-1977, XP002676853, ISSN: 1573-6903 abstract page 1971, column 2, paragraph 3 page 1972, column 2, paragraph 2 figures 1-2	5,6
A	----- JOHNSTON GRAHAM A R: "Medicinal chemistry and molecular pharmacology of GABA(C) receptors.", CURRENT TOPICS IN MEDICINAL CHEMISTRY AUG 2002 LNKD- PUBMED:12171579, vol. 2, no. 8, August 2002 (2002-08), pages 903-913, XP002676854, ISSN: 1568-0266 abstract page 905, column 2, paragraph 4 figures 2-5	1-36
A	----- BORMANN J J: "The 'ABC' of GABA receptors", TRENDS IN PHARMACOLOGICAL SCIENCES, ELSEVIER, HAYWARTH, GB, vol. 21, no. 1, 1 January 2000 (2000-01-01), pages 16-19, XP004189133, ISSN: 0165-6147, DOI: 10.1016/S0165-6147(99)01413-3 table 1	1-36
X	----- CHEBIB M: "GABAC receptor ion channels.", CLINICAL AND EXPERIMENTAL PHARMACOLOGY & PHYSIOLOGY NOV 2004 LNKD- PUBMED:15566397, vol. 31, no. 11, November 2004 (2004-11), pages 800-804, XP002676855, ISSN: 0305-1870 abstract page 801, column 2, paragraph 2 table 1 ----- -/--	5,6

INTERNATIONAL SEARCH REPORT

International application No

PCT/DK2012/050077

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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