A REVIEW ON THE PHYSIOLOGICAL AND PHARMACOLOGICAL INFLUENCE OF VASCULAR TONE IN CHOROIDAL AND CONJUNCTIVAL EYE TERRITORIES

AURELIAN ZUGRAVU\textsuperscript{1}, ISABEL CRISTINA VIORICA GHIŢĂ\textsuperscript{1*}, HORIA PĂUNESCU\textsuperscript{1}, LAURENȚIU COMAN\textsuperscript{2,6}, OANA ANDREIA COMAN\textsuperscript{1,10}, ION FULGA\textsuperscript{10}

\textsuperscript{1}Department of Pharmacology and Pharmacootherapy, Faculty of Medicine, “Carol Davila” University of Medicine and Pharmacy
\textsuperscript{2}Department of Physiology, Faculty of Pharmacy, “Carol Davila” University of Medicine and Pharmacy

*corresponding author: isabelghita@yahoo.co.uk
\textsuperscript{6}All authors have an equal contribution.

Abstract

This review analysed the literature of the period 2006 - 2015 on the nervous and humoral control of choroidal (including the vascularization of iris and ciliary bodies) and conjunctival vascular tone. Articles referring exclusively to pathological eye and to retinal vasculature were excluded. Anatomically, choroidal and conjunctival vascular territories are two distinct territories with extensive anastomosis. In both territories active substances interfering with the adrenergic, histamine and serotonin control have been assessed. The influences in the area of the ciliary arteries/choroidal vasculature of many other vasoactive substances were studied. For substances that have been studied in both vascular territories, there were differences between the two territories. Differences in vascular reactivity are likely due to the variability in density of the different types of adrenergic, histamine and serotonin receptors, respectively.

Introduction

Eye vascularization is well known by its particularities. It consists of the retinal vascular system and the uveal vascular system. Retinal circulation is a terminal vasculatization without arterial anastomoses. Choroidal circulation is a non-terminal type with many arterial and arteriovenous anastomoses [19]. Ocular vasculatization cannot be considered as one because of different origin from the external carotid artery and internal carotid artery respectively. It is generally considered that choroidal vasculature is derived primarily from the ophthalmic artery, a branch of the internal carotid artery. The groups of branches of the eye are represented by the following arteries: long ciliary arteries, short ciliary arteries, anterior ciliary artery, central retinal artery and muscular arteries [11]. Long posterior ciliary arteries pierce the sclera sideways of the optic nerve and splits into an upper and a lower terminal branch which anastomoses with the opposite corresponding branches and anterior ciliary arteries, forming a circle at the periphery of the iris - great arterial circle of the iris. This radial branches circle turns to the free edge of the iris where, through arterial anastomoses form the small arterial circle of iris; at this level originate the branches that supply the pupillary edge of the iris [3]. The posterior conjunctival arteries, branches from the arterial arches of the upper and lower eyelids that supply the conjunctiva, generate at the sclerocorneal limbus the palisades of Vogt. Anterior ciliary arteries supply blood to the bulbar conjunctiva and sclerocorneal limbus. They originate in the muscular branches of the ophthalmic artery. Prior to penetrate the eyeball, to 2 mm of limbus, ciliary arteries generate anterior conjunctival arteries, from
which anterior branches emerge and create a pericorneal plexus. At that level perforated branches of sclera ending at the great arterial arch of iris and recurrent branches that anastomose with posterior conjunctival arteries emerge [3]. Venous vascular bed has very complex patterns which do not closely follow the ramifications of the arterial system.

Considering the differences between the two vascular territories (choroidal/extraocular and intraocular ciliary arteries/iris and conjunctival) there may be differences in vascular control between the two territories.

The actual review evaluated the literature of the past 10 years on the influence of choroidal (including iris and extraocular and intraocular isolated ciliary arteries) and conjunctival vasculature by various endogenous and exogenous substances.

Materials and Methods

The research of physiological and pharmacological influence on the choroidal and conjunctival vasculature through literature review, involved the use of a complex "tag" shown below, in PubMed database, including scientific papers published between 2006 - 2015 in English and Romanian, not in the category of reviews. The used tag: (ocular OR eye) AND (ciliary OR conjunctival OR ophthalmic OR choroid OR choroidal) AND (arteries OR capillary OR veins) AND (humans OR bovine OR dogs OR cats OR canine OR feline OR rabbits OR mice OR mouse OR rats OR murine OR rodents) AND (vasodilatation OR vasodilation OR tone OR vasoconstriction OR blood flow).

Thus, initially 789 articles were identified. Of these, 706 were in English and one in Romanian; out of them 53 were literature review, 654 items remaining still considered.

Out of 654 articles, 653 articles have been identified to be related to the choroidal vasculature, including iris, ciliary and ophthalmic artery, and one related only to conjunctival vasculature. Out of 653 articles considered, 18 articles included references to conjunctival vasculature.

Finally, 27 articles were selected by removing the articles referring exclusively to retinal vasculature and those which analyses only the influence on vascularization of the pathological eye.

Results and Discussion

Figure 1 is showing the distribution per years of the articles originally selected, demonstrating a relatively constant interest of the scientists on the possibilities to influence the ocular vascularization.

The prostaglandins are involved in the control of ocular vascular tone. In the porcine long posterior ciliary arteries and other intraocular arteries (unspecified), all experiments performed in vitro, prostaglandin D₂ (PGD₂) acting on DP receptors and prostaglandin E₂ (PGE₂) acting on EP receptors, produced vasodilation [16]. There have not been found data, in the reviewed articles, regarding the vasodilator effects of prostaglandins on conjunctival circulation.

PGD₂ and PGE₂ caused vasoconstriction on porcine long posterior ciliary arteries, acting through thromboxane receptors (TP) [16]. Prostaglandin F₂α (PGF₂α) had vasoconstrictor effect on porcine ciliary arteries and long posterior ciliary arteries in vitro [15]. Also, Vysniauskiene et al., 2006 [26] showed that the PGF₂α produced vasoconstrictor effect on porcine ciliary arteries, effect inhibited by SQ29548 ([1S-[1α,2α(Z),3α,4α]]-7-[3-[[phenylamino]-carbonyl]-hydrazino]-methyl]-7-oxabicyclo-[2.2.1]-hept-2-yl]-5-heptenoic acid, a TP receptors antagonist) and by AL8810 (9α,15R-dihydroxy-1β-fluoro-15-(2,3-dihydro-1H-inden-2-yl)-16,17,18,19,20-pentanor-prosta-5Z,13E-dien-1-oic acid, a prostaglandin F receptor (FP) receptors antagonist). In ciliary arteries and long posterior ciliary arteries the same vasoconstrictor effect in vitro to travoprost and latanoprost (analogues of PGF₂α) and also for U46619 (9,11-diexoxy-9α,11α-methanoepoxy-prosta-5Z,13E-dien-1-oic acid, analogue of TXA₂) acting on TP and FP receptors was shown [16, 26]. Unoprostone isopropyl, latanoprost and tafluprost (PDF₂α receptor agonists) produced vasoconstriction in mouse ciliary arteries in vitro. This effect was mildly affected by the cyclooxygenases inhibitors - indomethacin - and it was not influenced by the administration of L-NAME (L-Nω-Nitroarginine treatment).
methyl ester), a non-specific inhibitor of NO synthase [1].

AL-12180 ((Z)-7-[(2R,3S,4R)-2-{(E,3R)-4-(3-chlorophenoxy)-3-hydroxybutyl-1-ethyl]-4-hydroxyoxolan-3-yl]-hept-4-enoic acid), a PGF analogue, produced vasoconstriction in the presence of extracellular K⁺ concentration of 80 mM in porcine short posterior ciliary arteries in vitro. Vasodilation was not recorded in any concentration, which may lead to the conclusion that AL-12180 acted only on TP receptors [22].

NO, formerly named EDRF (Endothelium-Derived Relaxing Factor), is a vasodilator factor that has as a secondary mechanism of action the transformation of GTP in cGMP [24]. According to Laspas et al., 2014 [18] NO donors produce vasodilation in the mouse ophthalmic arteries in vitro. The same authors showed that acetylcholine had a vasodilator effect on eye arteries in mice by a NO-dependent mechanism, this effect being simulated by the administration of sodium nitroprusside and blocked by the administration of L-NAME, except for the knockout mice for the gene encoding eNOS (endothelial NO synthase).

L-NMMA (L-\textenit{N}^{\textit{i}}-monomethyl Arginine) is a vasoconstrictor by nonspecific blockade of NO synthase. It produced vasoconstriction on human choroidal arteries through a NO-dependent mechanism in vivo [23].

H₂S donors, AP67 ((4-methoxyphenyl) pyrrolidin-1-yl phosphinodithioic acid) and AP72 ((4-methoxyphenyl)-piperidin-1-yl phosphinodithioic acid) assessed on bovine posterior ciliary arteries in vitro, arteries pre-contracted with phenylephrine, produced vasodilation [17].

The mechanism of vasodilation by AP 67 involves, beside H₂S, a prostaglandin component (is inhibited by flurbiprofen) also an ionic component by activating the ATP-sensitive potassium channel (K_{ATP}) channels (is inhibited by glibenclamide) and the involvement of NO (is inhibited by L-NAME). In case of AP72, prostaglandins are not involved but the H₂S, NO and K_{ATP} involvement was demonstrated.

On the bovine posterior ciliary arteries, considering the same method (arteries pre-contracted by phenylephrine in vitro) Chitnis et al., 2013 [4] showed that GYY4137 (p-(4-methoxyphenyl)-p-4-morpholinylphosphinodithioic acid), a donor of H₂S, produced vasodilation, by a mechanism involving the prostaglandin system, the administration of flurbiprofen facilitating GYY4137 vasodilator effect. Also, the same authors showed that inhibition of H₂S by aminoxyacetic acid and proparglyglycine and blocking of K_{ATP} by glibenclamide diminished the GYY4137 vasodilator effect. In addition, it was shown that the NOS inhibitor, L-NAME, did not influence the vasodilator effect of GYY4137 in the respective experiment [4].

An endothelial factor involved in blood vessels relaxation in the rat ciliary arteries territory in vitro is EDHF (Endothelium-derived hyperpolarizing factor). EDHF is actually a mechanism of vascular relaxation with an endothelial starting point than a substance itself, which has an independent action of blocking the pathway of prostaglandin and NO synthesis and whose action can be evidenced experimentally by administering acetycholine in the presence of indomethacin and L-NAME on arteries precontracted with norepinephrine. The mechanism of the vasodilator effect of EDHF could consist on influencing the K_{Ca} channels sensitive to charybdoxin (intermediate and large-conductance calcium-activated potassium channels blocker) and iberiotoxin (large-conductance calcium-activated potassium channels blocker) but not to apamin (small conductance calcium-activated potassium channels blocker) [10].

Wagenfeld et al., 2013 [27] showed that in the rat eye arteries in vitro, the reactive oxygen species (ROS), which can be considered a part of EDHF, dilated vessels with endothelium exposed during 5 seconds (s) and 20 s. In case of vessels without endothelium, the administration of ROS for 5 s produced vasodilation while exposure to ROS for 20 s produced vasoconstriction.

Wagenfeld et al., 2014 [28] tested the influence of the cells membrane potential of the vascular smooth muscle in vitro on the vasodilator and vasoconstrictor effects of ROS. At a resting potential of -60 mV, on ophthalmic artery in mice, ROS produced vasodilation, which was attenuated by blocking of the Na⁺/K⁺-ATP-dependent pump (by ouabain). At a potential of -41mV ROS caused vasoconstriction, abolished by KBR7943 (2-[4-[(4-nitrophenyl)-methoxy]-phenyl]-ethyl carbamimidothioic acid, monomethanesulfonate), an ion exchanger blocker of NCX (sodium-calcium exchanger).

Adenosine dilated the bovine posterior ciliary arteries in vitro. ATP in the presence of a pretreatment with alpha, beta-methylene ATP (a molecule which is a purinergic receptors antagonist, but not a specific antagonist and is slowly metabolized) also had a vasodilatory effect in vitro. ATP, alpha, beta-methylene ATP and uridine 5’-diphosphate produced vasoconstriction in vitro [30].

The administration of carbonic anhydrase inhibitors (acetazolamide, brinzolamide and dorzolamide) produced vasodilation which is dependent to NO release in porcine intraocular ciliary arteries territory in vitro [15]. Dorzolamide, in arteries pre-contracted with U46619 (analogue of TXA₂) showed a vasodilator effect in vitro, which did not involve carbonic anhydrase but involved vascular endothelium and NO [15].
treatment with ranitidine did not prevent histamine vasodilation, suggesting that the dominant receptor in conjunctival vessels was the H1 receptor.

Conjunctival administration of serotonin in vivo, acting on specific receptors, produced conjunctival and iridal vasoconstriction. Vasoconstrictor effect was of different intensities in the two territories, probably due to differences in density of serotonin receptors in the conjunctiva and iris [6].

Acetylcholine is not only a neurotransmitter but also a substance with non-synaptic endothelium activity. It is acting indirectly through NO, prostaglandins and so-called EDHF (which acts by Kcα channels sensitive to apamin and charybdoxin) [23]. Delaey et al. 2007 showed that in the bovine choroidal arteries, pre-contracted with norepinephrine, in vitro, the administration of acetylcholine produced a dose-dependent vasodilation up to maximal vasodilation at a 10 µM concentration. In the presence of L-NA (N0 Nitro-L-arginine, an inhibitor of NOS) vasoconstriction to norepinephrine was accentuated, but the vasodilator effect of acetylcholine in vessels pre-contracted with norepinephrine plus L-NA was unaffected. The vasodilator effect of acetylcholine was abolished in the presence of L-NA and indomethacin, in the presence of a high concentration of K+ (30 mM) and by mechanical removal of endothelium. TEA (tetraethylammonium, a nonselective K+ channel blocker) significantly reduced the vasodilator effect of acetylcholine. A similar effect was produced by administration of charybdoxin (intermediate and large-conductance calcium-activated potassium channels blocker) and apamin (small conductance calcium-activated potassium channels blocker).

Charybdoxin showed per se vasoconstrictor effects in choroidal arteries unlike apamin, which had no effect in this vascular territory [8].

Acetylcholine produced in vitro vasodilation in rat ciliary arteries pre-contracted with norepinephrine, a substance that produced vasoconstriction in the respective territory by acting on α1 receptors [10]. Also, Ziganshina et al., 2012 [30] showed the vasoconstrictor effect of norepinephrine on bovine posterior ciliary arteries in vitro.

Epinephrine 0.1% instilled in the eye of rats produced gradual vasoconstriction in iridal territory. In the conjunctival vessels a slight vasodilation occurred initially which was followed by vasoconstriction of lower intensity than that produced at the iris vessels level. Iris vessels treated with isoprenaline 0.00002% (a β1 and β2 adrenoceptor agonist) were unaffected, unlike conjunctival vessels, among which was a significant vasodilation.

Differences in the vascular reactivity of the two vascular territories of the eye (conjunctiva and iris), when treated with vasoactive amines (epinephrine, isoprenaline), supports the idea that the β2 adrenergic receptors are present only in the conjunctival...
vessels but not at the level of the iris, while α adrenergic receptors are present in both ocular vascular territories \textit{in vivo} [7]. Phenylephrine, an α₁ agonist, had vasoconstrictor effects on the bovine posterior ciliary arteries \textit{in vitro} [4], on the rabbit ciliary arteries \textit{in vivo} [9] and also produced vasoconstriction of choroidal arteries in humans \textit{in vivo} [23, 14], Laspas et al., 2014 [18] showed vasoconstrictor effect of phenylephrine on ophthalmic arteries in mice \textit{in vitro} and Gaynes et al., 2014 [13] showed vasoconstrictor effect on rabbit conjunctival arteries \textit{in vivo}. Isoprenaline produced vasodilation on iris and conjunctival arteries \textit{in vivo}, supporting that the β₂ adrenergic receptors are possible involved in this effect [7].

Arginine vasopressin acting on specific receptors caused vasoconstriction in rabbit choroidal territory \textit{in vivo} [2].

Sildenafil and tadalafil \textit{in vivo} increased the choroidal blood flow in humans by a cAMP-dependent mechanism [12]. On the bovine ophthalmic arteries pre-contracted by serotonin, anandamide ((5Z,8Z,11Z,14Z)-N-(2-hydroxyethyl) icosa-5,8,11,14-tetraenamide) and WIN 55212-2 ((3R)-2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenyl-methanone, monomethanesulfonate) produced dose-dependent vasodilation \textit{in vitro}. The mechanism of vasodilation involved the CB₁ receptors and endothelial-dependent vasodilator factors (acting through NO and the K_{Ca} channels) [21].

Table I is showing the influence of various substances in the eye vessels.

<table>
<thead>
<tr>
<th>Substance</th>
<th>(route of administration)</th>
<th>Effect</th>
<th>Species</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD₂ (in organ bath)</td>
<td>long posterior ciliary arteries via TP receptors</td>
<td>long posterior ciliary arteries via DP receptors</td>
<td>porcine*</td>
<td>[16]</td>
</tr>
<tr>
<td>PGE₁ (in organ bath)</td>
<td>long posterior ciliary arteries via TP receptors</td>
<td>long posterior ciliary arteries via EP receptors</td>
<td>porcine*</td>
<td>[15]</td>
</tr>
<tr>
<td>PGF₁α (in organ bath)</td>
<td>long posterior intraocular ciliary arteries via FP receptors</td>
<td></td>
<td>porcine*</td>
<td>[16, 26]</td>
</tr>
<tr>
<td>Travoprost (in organ bath)</td>
<td>ciliary arteries / long posterior intraocular ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[16, 26]</td>
</tr>
<tr>
<td>Latanoprost (in organ bath)</td>
<td>ciliary arteries / long posterior intraocular ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[16, 26]</td>
</tr>
<tr>
<td>U46619 (in organ bath)</td>
<td>ciliary arteries / long posterior intraocular ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[16, 26]</td>
</tr>
<tr>
<td>Unoprostone isopropyl (in organ bath)</td>
<td>ciliary arteries</td>
<td></td>
<td>mice*</td>
<td>[1]</td>
</tr>
<tr>
<td>Latanoprost (in organ bath)</td>
<td>ciliary arteries</td>
<td></td>
<td>mice*</td>
<td>[1]</td>
</tr>
<tr>
<td>Tafluprost (in organ bath)</td>
<td>ciliary arteries</td>
<td></td>
<td>mice*</td>
<td>[1]</td>
</tr>
<tr>
<td>AL-12180 (in organ bath)</td>
<td>short posterior ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[22]</td>
</tr>
<tr>
<td>NO as nitroprusside (in organ bath)</td>
<td>Ophthalmic arteries</td>
<td></td>
<td>mice*</td>
<td>[18]</td>
</tr>
<tr>
<td>L-NMMA (intravenously)</td>
<td>choroidal arteries</td>
<td></td>
<td>human#</td>
<td>[23]</td>
</tr>
<tr>
<td>AP67 (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[17]</td>
</tr>
<tr>
<td>AP72 (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[17]</td>
</tr>
<tr>
<td>GYY4137 (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[4]</td>
</tr>
<tr>
<td>ROS (in organ bath)</td>
<td>ophthalmic arteries in 5s and 20s administration</td>
<td></td>
<td>rats*</td>
<td>[27]</td>
</tr>
<tr>
<td>ROS (in organ bath)</td>
<td>denudated ophthalmic arteries in 5s administration</td>
<td></td>
<td>rats*</td>
<td>[27]</td>
</tr>
<tr>
<td>ROS (in organ bath)</td>
<td>ophthalmic arteries, at resting transmembrane potential</td>
<td></td>
<td>mice*</td>
<td>[28]</td>
</tr>
<tr>
<td>Adenosine (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[30]</td>
</tr>
<tr>
<td>ATP=alpha, beta-methylene ATP (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[30]</td>
</tr>
<tr>
<td>ATP (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[30]</td>
</tr>
<tr>
<td>Alpha, beta-methylene ATP (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[30]</td>
</tr>
<tr>
<td>Uridin 5’triphosphate (in organ bath)</td>
<td>posterior ciliary arteries</td>
<td></td>
<td>bovine*</td>
<td>[30]</td>
</tr>
<tr>
<td>Acetazolamide (in organ bath)</td>
<td>intraocular ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[15]</td>
</tr>
<tr>
<td>Brinzolamide (in organ bath)</td>
<td>intraocular ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[15]</td>
</tr>
<tr>
<td>Dorzolamide (in organ bath)</td>
<td>intraocular ciliary arteries</td>
<td></td>
<td>porcine*</td>
<td>[15]</td>
</tr>
<tr>
<td>Dorzolamide (conjunctivally)</td>
<td>ciliary arteries</td>
<td></td>
<td>rabbit#</td>
<td>[20]</td>
</tr>
<tr>
<td>Endotelina-1 (intravenously)</td>
<td>choroidal arteries</td>
<td></td>
<td>human#</td>
<td>[25]</td>
</tr>
</tbody>
</table>

Reference
On the other hand, the same author showed that, territory of conjunctival vessels. within the iris is more intense than that within the rats, Coman et al., 2008 [7] showed, for example, that in rats, in vivo, vasoconstriction caused by adrenaline within the iris is more intense than that within the territory of conjunctival vessels. On the other hand, the same author showed that, in vivo, isoprenaline produced a more intense vasodilation in the conjunctival territory than in the iridal territory. All this suggest that β adrenergic receptors density is higher in conjunctival vessels than at iris vessels level.

Also, regarding serotonin and histamine control, differences in the in vivo sensitivity between the two territories, have also been described. The histamine H1 receptors seem to be predominant in the conjunctival territory, while H2 receptors have a higher density within the iris. Most probably, the above described differences are not the only differences in the vasomotor control at different eye vascular territories; further research will bring new evidence of this hypothesis.

### References


