Use of isolated ocular arteries in vitro to define the pathology of vascular changes in glaucoma

Christine H Buckley, Patrick W F Hadoke, Colm J O’Brien

Glaucoma is a disease in which there is a progressive loss of visual field and a characteristic alteration in the appearance of the optic nerve head. While the exact pathogenesis of this condition remains unclear, a significantly increased intraocular pressure (IOP) has been clearly shown to lead to damage to the optic nerve head, and current treatment for glaucoma consists almost entirely of interventions which lower IOP. However, some patients with glaucomatous damage have an IOP within the normal range and in some cases of glaucoma reduction of IOP to normal values does not prevent the progression of the disease, indicating that factors other than an increased IOP may be involved in the development of this condition. A vascular role in the pathogenesis of glaucoma, as well as in other ocular diseases, has been suggested by the association between many systemic vascular diseases (including hypertension, migraine, diabetes, and peripheral vascular disease) and the presence of glaucoma. The systemic microcirculation and ocular blood flow, which are essential for the normal function of the optic nerve head, are both impaired in these disorders. Vascular factors may be of particular importance in normal pressure or low tension glaucoma which accounts for approximately one third of all glaucoma cases and in which IOP is normal. The presence of optic disc haemorrhages among patients with low tension glaucoma, indicating ischaemic optic microinfarction or vascular insufficiency to the optic nerve head, provides further support for a vascular role in the aetiology of this type of glaucoma.

Clarification of the vascular alterations during disease progression in the eye is complicated by the limited information available concerning the normal physiological control of ocular blood vessels and their role in maintaining the normal function of the eye. While the eye is one of the most highly perfused organs in the body, the reasons for this are unclear. In humans the ophthalmic artery branches from the internal carotid artery and then divides to form the ocular blood vessels with the blood supply to the eye separating into two vascular systems. As the ophthalmic artery crosses the optic nerve it divides into the central retinal artery and posterior and anterior ciliary arteries. The retinal vessels supply the inner layers of the retina whereas the uveal or ciliary vessels supply the optic nerve head, choroid, iris, and ciliary body.

In order to understand the role of ocular blood flow in both normal and pathological conditions, knowledge of the pharmacological control mechanisms involved in the ocular vascular bed is needed. While in vitro experiments using isolated ring segments of arteries, provide a useful approach to gaining this information, such work has been restricted by the dimensions of ocular vessels. Indeed, early work was limited to the use of smooth muscle strip preparations, a technique which is still used for the study of some larger arteries. This problem has been overcome by the development of the small vessel myograph by Mulvany and Halpern, which allows analysis of vessels with an internal diameter as small as 100 µm. Comparatively recent work using wire myograph systems has provided some insights into the regulation of ocular blood vessels (reviewed in Haefliger et al. and Brown and Jampol). Furthermore, a small amount of work has been performed using a perfused whole eye preparation. An additional technique which should prove to be important in this area is pressure myography. This technique allows investigation of the responses of vessels, similar in size to those used in the wire myograph, following cannulation and the intraluminal application of physiological salt solution. This produces an environment for the vessel which is closer to the in vivo situation than that produced in the wire myograph and, consequently, it has been demonstrated that vessels in a wire myograph are less sensitive to a variety of agonists than vessels in a pressure system or in vivo. Interpretation of the work performed using ocular vessels in vitro is difficult as many different ocular arteries and a wide variety of vasoactive agents have been utilised in varying degrees (Table 1). Furthermore, the inherent difficulties encountered in obtaining human tissue have led to the use of vessels from several different species of animal. This is important as there is well documented heterogeneity of vascular response between species, which complicates extrapolation of these results to the human.

This review aims to summarise the in vitro vasoactive studies carried out to date using various ocular arteries isolated from a number of different species, and to discuss the mechanisms that may be important in maintaining the vascular tone of these vessels. Finally, the review will consider how these systems may become impaired in glaucoma and the relevance of particular arteries and species used in glaucoma research.

Dilator responses

THE NITRIC OXIDE SYSTEM

Acetylcholine (ACh) induces relaxation in a large number of vascular smooth muscle preparations. In many arteries the dilator response is dependent on the presence of an intact layer of endothelial cells, and is due to the release of nitric oxide (NO) from the endothelium following stimulation of specific endothelial cell receptors (Fig 1). Several agonists and physical stimuli have now been shown to evoke endothelium dependent relaxations, although there is great heterogeneity in the responses. Many agents only elicit endothelium dependent relaxation in certain species,
or in specific vessels from certain species, and may cause contraction or endothelium independent relaxation in other preparations. Nitric oxide is formed from the amino acid L-arginine by the enzyme nitric oxide synthase (NOS), and its synthesis can be inhibited by analogues of L-arginine such as L-NG monomethyl arginine (L-NMMA) or L-NG nitroarginine methylester (L-NAME). Relaxation of vascular smooth muscle by nitric oxide is mediated by the activation of soluble guanylate cyclase leading to an increase in intracellular cyclic guanosine monophosphate (cGMP) levels. By using pharmacological inhibitors of the L-arginine–nitric oxide pathway, such as methylene blue, which inhibits guanylate cyclase, or haemoglobin, which binds to and inactivates nitric oxide, as well as NOS inhibitors, the mechanism of action of receptor agonists and the role of nitric oxide and the endothelium in the regulation of blood flow can be determined (Fig 1).

### Basal Nitric Oxide Release

In isolated quiescent bovine retinal arteries and porcine and human ophthalmic arteries inhibitors of nitric oxide formation induced endothelium dependent contractions. In addition, in the whole perfused porcine eye, L-NAME was found to decrease ophthalmic blood flow by 40%. Furthermore, in vivo studies have shown that intravenous infusion of NOS inhibitors results in a decreased blood flow in the choroïd of cats and of choroïd, ciliary body, and iris of dogs. Canine retinal blood flow, however, did not change significantly following treatment with L-NAME. In contrast, in miniature pigs, nitric oxide is continuously produced by cells of the retina and this was reduced by inhibition of NOS resulting in retinal vasoconstriction. Studies in rabbits have also demonstrated a 50% reduction in both uveal and retinal blood flow following inhibition of NOS. These studies indicate that nitric oxide is important in regulating both uveal and retinal blood flow under resting conditions, although differences between species may exist. Treatment with L-NMMA or removal of the endothelium have also been shown to augment 5-hydroxytryptamine (5-HT) evoked contractions of porcine ophthalmic artery. These results show that the endothelium continuously produces nitric oxide which maintains the ophthalmic circulation in a constantly dilated state and may have a protective role against vasospasm.

### Table 1  Different ocular arteries used in *in vitro* studies

<table>
<thead>
<tr>
<th>Species</th>
<th>Vessel</th>
<th>References</th>
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<tbody>
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<td>Rabbit</td>
<td>Ophthalmic artery</td>
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![Figure 1](image-url)  
**Figure 1** Mechanism of endothelium dependent relaxation showing the release of nitric oxide (NO), prostacyclin (PGI₂) and endothelium derived hyperpolarising factor (EDHF) from the endothelium. Agonists act on specific endothelial cell receptors and may stimulate the release of one or all mediators to induce smooth muscle relaxation. Abbreviations: Acetylcholine (ACh), histamine (Hist), endothelin-1 (ET-1), bradykinin (BK), substance P (Sub P), calcitonin gene related peptide (CGRP), nitric oxide synthase (NOS), arachidonic acid (AA), L-N⁰ monomethyl arginine (L-NMMA), L-N⁰ nitroarginine methylester (L-NAME), haemoglobin (Hb), glyceryl trinitrate (GTN), sodium nitroprusside (SNP), 3-morpholinosydnonimine (SIN-1), methylene blue (Meth blue), cyclic guanosine/adenosine monophosphate (cGMP/cAMP), guanosine/adenosine triphosphate (GTP/ATP), non-adrenergic non-cholinergic (NANC), muscarinic receptor (M), histamine receptor (H₁), endothelin B receptor (ET₉), bradykinin receptor (B₂).
AGONIST INDUCED NITRIC OXIDE RELEASE

ACh evoked relaxations in isolated bovine retinal arteries,18 porcine ophthalmic arteries,19 and human posterior ciliary and opthalmic arteries.20 21 relaxations were abolished on removal of the endothelium. In contrast, relaxations evoked by ACh in canine opthalmic and retinal arteries were not dependent on an intact endothelium but were mediated by the release of prostacyclin (PGL) from subendothelial tissues2 12 indicating that some heterogeneity between species exists. Endothelium dependent relaxations in response to histamine, bradykinin, and substance P have also been demonstrated in isolated bovine and canine retinal arteries.18 19 22 and porcine ciliary artery.17 18 20 21 The endothelium dependent relaxation elicited by ACh was completely abolished by atropine, methylene blue, and L-NMMA,25 26 indicating that the response was mediated by activation of endothelial muscarinic receptors stimulating the release of nitric oxide. In addition, in vivo experiments have demonstrated an increased choroidal blood flow in response to intravenous infusion of ACh which was reduced following co-infusion of the NOS inhibitor N Nito-arginine,56 suggesting a role for nitric oxide in the control of choroidal blood flow.

In the intact perfused porcine eye, bradykinin resulted in a concentration dependent increase in opthalmic flow which was completely prevented by L-NAME,16 suggesting that nitric oxide is the primary mediator of the vasodilatory response to bradykinin. In contrast, studies using isolated porcine ophthalmic and ciliary arteries demonstrated that the relaxing effect of bradykinin was markedly reduced, but not abolished, by L-NMMA,26 indicating that nitric oxide contributes only in part to the relaxation and a relaxing factor distinct from nitric oxide, possibly endothelium derived hyperpolarising factor, may also be involved. Furthermore, the bradykinin stimulated release of nitric oxide increased with decreasing vessel diameter in the porcine extraocular opthalmic circulation,26 indicating that endothelium mediated responses can be influenced by vessel size.

The endothelium dependent relaxation evoked by histamine in bovine retinal and human opthalmic arteries was mediated mainly by activation of endothelial H₁ receptors.19 20 In bovine retinal arteries, the relaxation was completely abolished by methylene blue and also partly blocked by indomethacin27 which may indicate that the response was mediated by both nitric oxide and a cyclo-oxygenase product such as PGI₂. In human arteries, however, the relaxation did not involve PGI₂ and was only partially blocked by L-NAME, the remainder of the response being sensitive to inhibition by an H₁ receptor antagonist.28 The dilator response to histamine in canine opthalmic artery depends on the anatomical location of the vessel; in the internal opthalmic artery the relaxation was independent of the endothelium and mediated by H₁ receptors located on the vascular smooth muscle.15 Histamine induced relaxation in the canine extracellular opthalmic artery, however, was partly endothelium dependent mediated by endothelial H₁ receptors causing the release of PGI₂ with the remaining response mediated by direct activation of smooth muscle H₁ receptors.17 These dilator actions, observed in response to histamine in bovine retinal and human and canine opthalmic arteries, are in marked contrast to the contractile responses seen in bovine ciliary and cat opthalmic arteries when stimulated with histamine.18 29 30 Nitric oxide has therefore been shown to relax both the endothelium both under basal conditions and following stimulation with agonists such as acetylcholine or bradykinin.

NEURONAL NITRIC OXIDE

In addition to mediating vasodilation following release from the endothelium, nitric oxide has also been implicated as a neurotransmitter responsible for the non-adrenergic non-cholinergic (NANC) vasodilator response in both the peripheral and central nervous systems.9 10 The oculcar circulation is innervated by nerves containing a number of neurotransmitters including noradrenaline and acetylcholine as well as a variety of neuropeptides.32 Using immunohistochemical techniques, the presence of NOS containing neurons has been demonstrated in ocular tissues of rat, rabbit, and human origin.53 54 In the rat, NOS activity is localised in peripheral parasympathetic nerve fibres derived from the pterygopalatine ganglion and supplying mainly the choroid and limbal vessels as well as in a number of retinal cell types.55 Studies using human eyes have also shown a wide distribution of NOS in the choroid.14 In support of a neurotransmitter role for nitric oxide in the ocular circulation, studies using transmural electrical stimulation of isolated arteries have shown that nerves innervating human and bovine long posterior ciliary arteries and also canine opthalmic arteries release nitric oxide which mediates vasodilatation.11 25 32

OTHER DILATOR AGONISTS

β Adrenoceptor antagonists are widely used in the treatment of glaucoma to lower IOP, although the mechanism involved in their therapeutic efficacy is not fully understood and effects on blood flow may also contribute to their effectiveness in glaucoma treatment. β Blockers, such as betaxolol and timolol, have been found to relax bovine retinal arteries precontracted with KCl and 5-HT1 and also porcine posterior ciliary arteries activated with KCl and the thromboxane mimetic U46619 by a mechanism not related to inhibition of adrenoceptors nor to the local anaesthetic properties of the drugs. However, owing to the similar relaxation properties of β blockers and Ca²⁺ antagonists, it was suggested that the relaxation of ocular arteries evoked by the β adrenoceptor antagonists was due to their Ca²⁺ antagonistic properties.11 36

The relaxing effect of Ca²⁺ antagonists has also been demonstrated in other studies using bovine retinal arteries precontracted with KCl, PGE₁,33 and ET-1,37 with KCl, but not noradrenaline, in canine opthalmic and long posterior ciliary arteries,34 45 and with aβ agonists in cat opthalmic arteries35 and porcine ciliary arteries contracted with ET-1.38 These studies indicate that the contractions to these agonists are dependent, at least in part, on an influx of extracellular Ca²⁺ through membrane potential operated calcium channels.

Some metabolites of arachidonic acid have been shown to elicit relaxation in ocular arteries. These include PGI₂ in bovine retinal and canine opthalmic arteries15 25 and prostaglandin E₁ (PGE₁) in canine opthalmic artery.16 In bovine retinal arteries, however, PGE₁ has been shown to evoke concentration dependent contractions.37 PGI₂ can also be released from the endothelium, in response to chemical or mechanical stimulation, mediating relaxation through the activation of adenylate cyclase and the formation of cAMP.

Constrictor responses

ADRENOCEPTOR AGONISTS

Canine opthalmic and long posterior ciliary arteries have been shown to contract in response to 5-HT, noradrenaline, adrenaline, and the selective α₁ agonist, phenylephrine but not to α₂ selective agonists.41 42 In addition, some heterogeneity in the responses to the agonists was
described in canine long posterior ciliary arteries, depending
on the exact anatomical location of the vessel. 63
Similar studies using ophthalmic artery rings from the
rat have concluded that in this artery functional histamine,
5-HT, and β1 adrenergic receptors mediating contraction
are present whereas α1 and β adrenoceptors are absent. 46
Again differences in sensitivity to these agonists existed
between proximal and distal segments of the artery.
Isolated human posterior ciliary arteries and ophthalmic
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shown to contract in response to 5-HT, noradrenaline,
and re-exposure to the peptide resulted in a marked tachyphylaxis \(^{19,26}\) which may be due to a downregulation of endothelin receptors. This may provide a protective mechanism against prolonged exposure to ET-1. In bovine and porcine vessels the ET-1 induced contraction was partly reversed by voltage operated calcium channel blockers \(^{29,40}\); in human ophthalmic artery, however, calcium antagonists had no effect on the contractile response to ET-1. \(^{19}\) In some species, therefore, ET-1 induced contraction is mediated in part by an influx of extracellular calcium through

**Table 2  Responses of ocular arteries to dilator agonists**

<table>
<thead>
<tr>
<th>Agonist</th>
<th>Artery</th>
<th>Species</th>
<th>Endothelium dependent</th>
<th>Receptor</th>
<th>Mediator</th>
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<td></td>
<td>feline</td>
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<td></td>
<td>Ciliary:</td>
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<td>H(_1)/H(_2)</td>
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<td>Ca(^{2+}) antagonism?</td>
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<td>canine</td>
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Muscarinic/nicotinic receptor (M/N), nitric oxide (NO), prostacycin (PGI\(_1\)), bradykinin receptor (B\(_2\)), endothelium derived hyperpolarising factor (EDHF), histamine receptor (H\(_1\)/H\(_2\)), prostaglandin E\(_2\) (PGE\(_2\)), sodium nitroprusside (SNP), 3-morpholinosydnonimine (SIN-1), glyceryl trinitrate (GTN).
Table 3  Responses of ocular arteries to constrictor agonists

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<th>Receptor</th>
<th>Mechanisms</th>
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<td>Ciliary:</td>
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α Adrenoceptor (α1/α2), 5-hydroxytryptamine (5-HT), 5-HT receptors (5-HT1/3), histamine receptor (H1), angiotensin II receptor (AT1), phospholipase C (PLC), prostaglandin (PG), thromboxane A2 (TxA2).

Voltage operated calcium channels and partly through other mechanisms such as the activation of phospholipase C and release of intracellular calcium. The porcine extraocular ophthalmic circulation shows some degree of heterogeneity in the response to ET-1 in that the sensitivity to the peptide increased with decreasing vessel diameter. This further demonstrates that the importance of endothelium dependent responses increases as the blood vessel diameter decreases, indicating a crucial role for these mechanisms in the regulation of the ophthalmic microcirculation. Although in isolated ocular arteries, from bovine, porcine and human origin, ET-1 only causes contraction, in the isolated perfused porcine eye vasodilatation is observed at low doses of ET-1 followed by a significant vasoconstriction at higher concentrations demonstrating the presence of both ET\textsubscript{a} and ET\textsubscript{b} receptors mediating constriction and dilatation respectively.

**NEURONAL REGULATION**

Regional variation exists in the innervation of the ocular circulation. Whereas several nerves regulate uveal blood flow, and extraocular and choroidal vessels are regulated by autonomic innervation, vasoactive nerves do not regulate blood flow through the retina or optic nerve. In the latter, autoregulatory mechanisms exist to maintain a relatively constant blood flow via mechanisms independent of changes in IOP or vascular perfusion pressure, although the presence of both adrenergic and cholinergic binding sites has been demonstrated. The role of these binding sites remains unclear and, in contrast with the investigations into NANC innervation, there appear to be no studies in which isolated vessels have been electrically stimulated to investigate the role of neurotransmitters.

**Vascular alterations in glaucoma**

A role for vascular alterations in glaucoma, proposed to explain deficiencies in the pressure theory, (reviewed in Flammer) suggests that impaired vascular responses (for example, increased vasospasm) combine with systemic alterations (for example, hypotension) to reduce ocular blood supply. This is supported by the observation that many glaucoma patients have widespread cerebrovascular and cardiovascular disease and increased vasospasm (indicated by the prevalence of migraine and a Raynaud’s-like peripheral circulation). Alterations in blood coagulation may also play a role in some types of glaucoma with evidence of an increase in both platelet adhesiveness and spontaneous platelet aggregation reported, as well as an increase in plasma viscosity. Such abnormalities have also been found to be associated with myocardial infarction and cerebrovascular disease. Furthermore, increased plasma levels of ET-1 are seen in some types of glaucoma and also in systemic cardiovascular (atherosclerosis, pulmonary hypertension, and chronic heart failure) and vasospastic (Raynaud’s phenomenon and variant angina) disorders.

The mechanisms underlying these vascular abnormalities remain unclear but may be due to an impaired release of NO from the vascular endothelium. Autoregulation, which may be regulated by local dilator and constrictor systems, has been demonstrated in the vessels of the retina, choroid, and optic nerve head. This ensures that an increase in intraocular pressure, plus a decrease in perfusion pressure, stimulates a drop in vascular resistance which maintains an unchanged blood flow. In some conditions, autoregulation may be reduced, possibly as a result of endothelial cell dysfunction and a similar situation is seen in some cases of glaucoma in which autoregulation of retinal and optic nerve head blood flow is impaired. It has been shown that blood flow velocity in the central retinal, ophthalmic, and short posterior ciliary arteries is reduced in some cases of glaucoma. As the posterior ciliary artery provides the main source of blood supply to...
the optic nerve head, reduced blood flow in this vessel may lead to glaucomatous optic nerve damage.

Use of isolated vessels in investigating the pathology of glaucoma

The relatively small amount of work performed using ocular blood vessels in vitro is disparate using a variety of species, arteries, and vasoactive compounds. Little in depth analysis of signal transduction mechanisms has been performed in these arteries but such data that are available demonstrate heterogeneity not only between species and vessels, but also between different parts of the same vessel (Tables 2 and 3). Indeed, functional responses may alter markedly as the diameter of a vessel changes. This emphasises the need to select the most suitable species for use as vessel donors (when human vessels are unavailable), the need for certainty that the vessel chosen is relevant to the aims of the research, and care in ensuring that the vascular rings selected are taken from the same anatomical location each time.

The use of retinal vessels in vitro is attractive as this allows investigation of phenomena (for example, vaso-spasms) which have been observed in vivo. However, it may be that such alterations are more important, and more prevalent, in ciliary or choroidal blood vessels. Use of choroidal blood vessels, while possible in larger animals, is restricted by the difficulties encountered in the dissection of these vessels and the relatively small contractions they produce in vitro. In addition to being highly relevant to glaucoma research, ciliary arteries are possibly the most convenient for use in myograph systems as they are relatively simple to dissect, are a good size to handle and produce strong contractile responses. Anterior ciliary arteries will provide information on the regulation of blood flow to the ciliary processes at the front of the eye while the short posterior ciliary arteries provide information about blood flow to the optic nerve head. The regulation of blood flow to the entire eye may be usefully investigated using the ophthalmic artery but care is needed to ensure that the same portion of the vessel is taken for each experiment. It should also be remembered that altered venous regulation may contribute to changes which cause ischaemia in the eye, suggesting that assessment of venous, as well as arterial, function should be investigated in vitro. The feasibility of such investigations has been demonstrated by the use of bovine isolated aqueous veins in a wire myograph system. This may have the further advantage of allowing investigation of the outflow channels for aqueous humour which appear to be regulated in a manner similar to blood vessels. The practical and ethical difficulties encountered in obtaining human tissue, and the consequent use of arteries from experimental animals, have led to the need to extrapolate results to the human condition. The obvious need to use animals with an ocular circulation as similar to the human as possible is tempered by the cost and ethical concerns encountered with using non-human primates. Canine, bovine, and porcine eyes are the most useful for isolated vessels studies (Table 1) as the ophthalmic, ciliary, and retinal arteries are all of a suitable size for use in the myograph. Of these, the porcine eye is probably most similar to the human and this is reflected by the increasing body of work performed with both isolated vessel and perfused eye techniques from this source. Furthermore, the relaxant (Table 2) and contractile (Table 3) responses evoked in porcine vessels are similar (although not identical) to those which have been demonstrated in isolated human vessels. A final consideration, however, reflects the availability (and continued development) of animal models of glaucoma. In such cases, the identification of pathological alterations in vascular function will be of immense interest even if the animal (for example, the rabbit) has an ocular circulation that is not identical to that in the human.

Conclusions

This review has described the important insights into the maintenance of ocular flow which have been gained using in vitro investigation. This work demonstrates the heterogeneity of responses between species, vessels, and different parts of the same vessel, indicating that the importance of nervous and autoregulatory control mechanisms varies between vessels and that the endothelium plays a key role in the maintenance of blood flow. Equilibrium between constrictor and dilator compounds released from the vascular endothelium may be central to the control of normal flow in the eye and derangements in this system may be implicated in the development of glaucoma and other ocular vascular diseases. Continued use of myographic techniques, coupled with the development of animal models, may enable clarification of the vascular changes in glaucoma. Furthermore, this technique will allow investigation of the vascular effects produced by therapies used in the treatment of this condition.

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References

Use of isolated ocular arteries in vitro to define the pathology of vascular changes in glaucoma

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