Effects of Extracellular pH on Agonist-Induced Vascular Tone of the Cat Ophthalmociliary Artery

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Purpose. To test whether changes in extracellular pH (pHe) in an in vitro preparation of the cat ophthalmociliary artery affect passive tone and agonist responses and whether the endothelial cells are mediators of any pH-induced effect. This will determine the ability of the ophthalmociliary artery to influence retinal and choroidal blood flow in response to metabolic stimuli.

Methods. The isometric tension generated by isolated ring segments preactivated by prostaglandin F2α (PGF2α), noradrenaline (NA), or 40 mM K+ was measured as the pHe of the bathing solution was changed stepwise from 6.0 to 8.0 by adjusting the bathing bicarbonate concentration in preparations with and without functioning endothelial cells.

Results. PGF2α produces a concentration-dependent contraction that is insensitive to an alkaline shift from control pHe (7.4) in the bathing medium. For acidic shifts to pHe 7.0, there is no significant change in the magnitude of the PGF2α contraction, whereas at pHe 6.0, the PGF2α contraction is reduced to 23 ± 4% (n = 23) of its value at pHe 7.4. Threshold response concentration remains unaffected. Deliberate damage to the endothelial cells does not significantly affect the magnitude of the 10^{-5} M PGF2α response at pHe 7.4 nor the effect of acidic pH on this response. The 10^{-5} M NA response is reduced in a graded fashion to acidic shifts to pH 7.0 and 6.0 (40 ± 4% [n = 23]) and also to alkaline shift to pH 8.0 (22 ± 5% [n = 23]) when compared to the induced tension at pHe 7.4. For the acidic shift only, endothelial cell damage causes a further significant reduction in the NA response to 20 ± 3% (n = 5). For vessels contracted with K+-Krebs solution, there is a small but significant reduction in response at pHe 6.0 to 84 ± 6% (n = 25), whereas for pHe 8.0 there is a much larger reduction to 45 ± 5% (n = 24). All pHe-induced relaxations of K+ are endothelium independent. Passive tension is unaffected by all pHe manipulations.

Conclusions. Vessel responses to vasoactive agents are selectively mediated by pHe changes. Major acidic shifts cause reduced responses (relaxation) to NA, PGF2α, or K+, whereas only vessels preactivated with NA and K+ relax to alkaline shifts. This implies that NA or K+ induced vascular responses are maximal close to neutral pHe with major shifts from neutrality in either the acidic or alkaline direction causing a reduced response. These results imply that the ophthalmociliary artery probably does not play a major role in controlling ocular blood flow in response to pHe changes within the normal metabolic range, but it may become important in ischemic conditions. Invest Ophthalmol Vis Sci. 1994;35:998-1007.

Regulation of intracellular and extracellular hydrogen ion concentration (pH) within narrow limits has classically been thought of as a prerequisite to neural tissue homeostasis. The intracellular–extracellular ionic conductances and enzyme activities are particularly sensitive to changes in local pH. There are several reports showing that there is a [H+] gradient between intracellular and extracellular compartments, with pH values ranging from 7.12 to 7.37 for a pHe value of 7.4.1-4 The belief that neural tissue maintains a ceaseless struggle to retain both extracellular pH (pHe) and intracellular pH (pHi) constant has been widely held. Recently, however, this simplistic homeostatic notion has shifted as it has become increasingly apparent that transient local pHe and pHi changes...
occur in response to many stimuli, including neural stimulation and receptor induced-vascular smooth muscle contraction. These pH shifts are usually fairly small, about 0.2 to 0.4 pH units. They are not just compensated for, but in fact act as regulators themselves to control subsequent biochemical events within neural and vascular tissue. Thus, pH fluctuations are now perceived to play an important regulatory role both intracellularly and extracellularly, linking neural, glial, and vascular tissue function and acting to control enzyme function and ion channel conductance.

In addition to these pH-induced changes during normal neural function, there are pathologic situations in which larger tissue pH changes occur. For example, complete ischemia can reduce tissue pH to about 6.0 in 15 minutes. Under these circumstances, there is evidence that vascular reactivity to vasoactive substances is altered in the brain and that over a wide range of acidic pH changes, from 7.4 to 5.0 arterioles relax. For milder conditions, such as metabolic and respiratory acidosis and alkalosis as well as partial ischemia, there may also be considerable changes in tissue pH that may affect vessel reactivity.

The ophthalmociliary artery has a dual role as a supply vessel to the uveal and retinal circulations and to the tissue of the optic nerve directly through its own capillary bed. Whether it passes partly within the neural and glial tissue of optic nerve or along the outside of the optic nerve depends on the animal species. Its location at the entry point to the eye places it in an important strategic position for mediating total ocular blood flow because its diameter and hence vascular resistance are changed by many vasoactive factors. Normally, control of local blood flow to neural tissue is a complex function of four factors: autonomic innervation, blood-borne factors, blood pressure induced myogenic responses, and local tissue released mediators. The relative balance of these factors differs between vascular beds and under different conditions in the one vascular bed. For the ophthalmociliary artery, which is autonomically innervated, it is possible that only the first three of these plays a role in controlling diameter, and hence blood flow, in the ophthalmociliary artery. However, conditions such as tissue ischemia caused by local or systemic factors would be expected to result in lowered tissue pH and PO₂ values. Certainly, the demonstration of acidic shifts in pH of 0.2 units in the rat optic nerve in response to increased neuronal activity raises the possibility of tissue-mediated pH being one factor controlling ophthalmociliary artery blood flow.

We have recently reported the effect of changing oxygen tension around the ophthalmociliary artery in vitro and have demonstrated that noradrenaline responses depend on the ambient oxygen tension and are probably mediated in some cases by the endothelial cells lining the vessel lumen, although these effects only occurred at the extreme ranges of PO₂. In this paper, we investigate the parallel hypothesis that agonist responses may be controlled partly by pH in an in vitro preparation of the cat ophthalmociliary artery and explore the possible mediating role played by the endothelial cells in such control. With these data, we hope to understand better the importance of the ophthalmociliary artery in complex and hierarchical control of blood flow to all regions of the eye.

The two agonists used, noradrenaline (NA) and prostaglandin F₂α (PGF₂α), are chosen for their likely but different roles as vasoconstrictors in the ophthalmociliary artery. NA is probably released in vivo by the local autonomic endings, and previous publications have already confirmed the vasoconstrictive effect of NA mainly through α₁ adrenergic receptors on cat, dog, human, and monkey ophthalmociliary artery. PGF₂α is a putative vasoconstrictive autocoid possibly released locally by neural tissue or by mural cells lining the vessel lumen in vivo in response to local stimuli. Thus, the generating stimuli and mechanisms of action of these two agonists on vascular smooth muscle may be expected to differ. There is already evidence that the monkey and human ophthalmociliary arteries are responsive to PGF₂α.

Any change in pH will, of course, induce some unknown change in pH of the autonomic nerve endings, endothelial cells, and smooth muscle cells in an isolated preparation of the ophthalmociliary artery. It is possible to alter pH directly, leaving pH unchanged using the elegant and verified method of addition of ammonium salts to the bathing medium. However, we chose instead to alter pH by adjusting the bicarbonate concentration of the bathing solution because this mimics more closely the in vivo tissue or blood changes in pH that naturally occur.

METHODS
Surgery
All experiments conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research. Nine adult domestic cats (1.5 to 3.0 kg) were deeply anesthetized (2.5% halothane) and heparinized (1000 U/cat). After disinserting the extraocular muscles, both eyes were carefully enucleated ensuring that a maximum length of optic nerve (circa 12 mm) was removed, after which the cat was killed with an anesthetic overdose.

Myograph System and Organ Bath
The myograph has already been described. A small ring segment of the ophthalmociliary artery was mounted in a temperature-controlled organ bath via...
two parallel tungsten wires (gold plated) passed through the vessel lumen, one of which was mechanically connected to an isometric force transducer and the other to a hydraulic microdrive system driven in the X direction. One of six bathing solutions (composition detailed below) was added to the 5 ml organ bath through a hydrostatic pressure head. Bath temperature was maintained close to 37°C. All solutions were equilibrated with carbogen (95% O₂, 5% CO₂) by continuous slow bubbling before and after adding to the bath. Bath solution temperature and PO₂ were continuously monitored and were displayed both digitally and on a chart recorder.

Isolated Vessel Preparation

The enucleated eye was placed in Na+-Krebs solution equilibrated with carbogen at 4°C for not less than 1 hour. The ophthalmic artery and connective tissue were carefully dissected free of the optic nerve. Three to four vascular ring segments (1 to 1.5 mm length) were cut from the middle section of each ophthalmic artery, average diameter 525 ± 10 μm (n = 9), and individually mounted in the myograph system under microscope control (magnification ×40). All handling of the vessel was by means of forceps and connective tissue until the vessel was mounted on the tungsten wires, after which the connective tissue was carefully dissected away. The bath was filled with Na+-Krebs bathing solution and the vessel segment left to equilibrate for no less than 30 minutes under zero tension. As the passive or active tension (measured in mN/mm) varied, the resulting change in distance between the two tungsten wires was directly measured by means of a micrometer eyepiece. The two tungsten wires were sufficiently rigid to remain parallel throughout all active and passive tension changes. The isometric tension output from the transducer was amplified, filtered (DC to 3 Hz), and recorded on a 6-channel chart recorder. The overall compliance of the recording system, determined by measuring the distance between the tungsten wires before and after a vessel, was subjected to a K+-Krebs activation at the appropriate pH value, followed after washout by a dose-response curve to either NA or PGF₂α in the normal Na+-Krebs solution. During the tonic phase of the 10⁻⁵ M response to NA or PGF₂α, the relaxation effect of ACh was determined (10⁻⁴ to 10⁻³ M) to confirm endothelial cell function. After washout, the vessel segment tension returned to baseline. The bath solution was then replaced by the two pH solutions in random order, followed by a return to control pH. At each pH value, the vessel segments were challenged sequentially with a K+-Krebs activation at the appropriate pH value, followed after washout by a dose-response curve to either NA or PGF₂α in the normal Na+-Krebs solution at the same pH value. In a subset of the experiments, the effect of endothelial cell damage on these pH-induced responses was determined. Selec-
tive endothelial cell damage was achieved by bubbling gas (95% O₂, 5% CO₂) through the lumen for 1 minute, and loss of endothelial cell function was confirmed by the failure of ACh to produce a relaxation response on a preactivated vessel. For five vessel segments, the effect of pH changes on resting isometric tension was also determined. The possible role played by release of endogenous prostaglandins in mediating pH-induced responses was tested by preincubation of the vessels in indomethacin (10⁻⁵ M) for six vessel segments.

Calculations and Statistical Analysis
All dose-response curves for each agonist are normalized to the value of the response produced by 10⁻⁵ M agonist in Na⁺-Krebs solution. For the initial three animals, three ring segments each, two two-way analyses of variance were performed to test the hypothesis that the effect of pH on both NA or PGF₂α activated vessels was different between animals when compared to repeated measurements for ring segments from the same animals. The two calculated tables and the subsequent F tests (accepting a level of significance of P < 0.05) showed that the between-animal variance was not significantly different from the within-animal variance (P > 0.25). This enables the results from all ring segments from all animals to be pooled. Results are thus presented as the grand average for all the vessel segments ± standard errors. Tests for the differences of mean values are performed using a two tailed Student’s t-test for which a P value of < 0.05 is accepted as significant.

RESULTS
pH Effect on Vessels Preactivated With PGF₂α
In Figure 1, raw data are presented to show a time sequence of K⁺-Krebs activations and PGF₂α dose-response curves in different pH solutions. First, the 40 mM K⁺-Krebs contraction at pH 7.4 is shown (K) followed by a washout (W). The ensuing PGF₂α dose response in Na⁺-Krebs solution from 10⁻¹⁰ to 10⁻⁵ M produces a strong contraction for concentrations of 10⁻⁶ to 10⁻⁵ M. Lower concentrations to which the vessel did not respond are not shown. This is followed by a washout (W), after which the tension returns to baseline. The whole procedure is repeated for pH values of 6.0 and 8.0 and followed by a return to the control pH of 7.4. It is apparent that PGF₂α produces a graded contraction insensitive to the alkaline shift in the bathing medium. However, for the acidic shift, the magnitude of the PGF₂α contraction is considerably reduced, with the threshold concentration unaltered. Thus, acidic conditions reduce the contraction produced by PGF₂α. In contrast, the K⁺-Krebs response is slightly reduced for the acidic shift, whereas the alkaline shift causes a large reduction in response. There is no change to baseline passive tension with pH shifts. The return to control pH 7.4 at the end of this sequence demonstrates the stability of the vessel and indicates that these are truly pH-induced changes. These observations are confirmed when the complete data sets from 23 separate segments are averaged. The average magnitude of the 10⁻⁵ M PGF₂α response as a percentage of the 40 mM K⁺-Krebs contraction is 151.9 ± 13.7% (n = 23). The average cumulative con-
traction dose response curves for PGF₂α for vessel segments for the three pH values are shown in Figure 2A (pHe 7.4, filled circles; pHe 6.0, open circles; pHe 8.0, open triangles). Tension responses are normalized to that produced by 10⁻⁵ M PGF₂α in the control solution (Na⁺-Krebs pH 7.4). The effect of pH shifts was asymmetric, with the acidic shift causing a reduction to 23 ± 4% (n = 23) of the control response (100%), whereas the alkaline shift caused no significant change with the response being 110 ± 14% (n = 22) of the control response. Deliberate damage to the endothelial cells did not significantly affect the relative magnitude of the 10⁻⁵ M PGF₂α response (135.6 ± 14.6% [n = 4]) measured as a percentage of the 40 mM K⁺-Krebs contraction. Neither did it affect the pH-induced effects on the PGF₂α response. Equivalent curves for vessels with damaged endothelial cells are shown in Figure 2B. None of the dose-response curves were significantly different from those from normal vessels (see also Tables 1 and 2). For all three pH conditions, the dose-response curves for PGF₂α had similar concentration thresholds of between 10⁻⁷ M and 10⁻⁶ M.

Preincubation with 10⁻⁵ M indomethacin also had no effect on these pH-induced effects.

**pH Effect on Vessels Preactivated With NA**

The responses for NA are smaller than those for PGF₂α, with the average percentage response at 10⁻⁵ M NA being 105.1 ± 13.3% (n = 23) of the 40 mM K⁺-Krebs contraction. Normalized cumulative dose-response curves for NA for the three pH conditions are plotted for vessel segments with and without normal endothelial cell function, respectively, in Figure 3 (pHe 7.4, filled circles; pHe 6.0, open circles; pHe 8.0,

### Table 1. Comparison of the Effect of pH 6.0 on the Contractions Induced by PGF₂α, NA, and K⁺-Krebs in Vessels With and Without Endothelial Cell Damage

<table>
<thead>
<tr>
<th>Vasoconstrictor</th>
<th>Endothelium (%)</th>
<th>Damaged Endothelium (%)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGF₂α</td>
<td>23 ± 4 (23)</td>
<td>7 ± 4 (4)</td>
<td>NS</td>
</tr>
<tr>
<td>NA</td>
<td>40 ± 4 (23)</td>
<td>20 ± 3 (5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>K⁺</td>
<td>84 ± 6 (25)</td>
<td>62 ± 2 (4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are normalized to control contractions at pH 7.4 (means ± SE, values in parentheses are number of samples).
pH Effect on Ophthalmociliary Artery Response

FIGURE 3. The average cumulative dose-response curves for NA for ring segments of the ophthalmociliary artery, (A) for vessels with a functioning endothelium, and (B) for vessels with the endothelium deliberately damaged. E% represents the isometric tension expressed as a percentage of the tension generated by 10^{-5} M NA pH 7.4. The abscissa is the applied NA concentration as log ([M]). pH 7.4 (filled circles), pH 8.0 (open triangles), and pH 6.0 (open circles) (n = 23).

open triangles). Both the acidic and alkaline shifts cause a reduced NA response, with the 10^{-5} M NA response being 40 ± 4% (n = 23) and 22 ± 5% (n = 23), respectively, of the control value at pH 7.4. Deliberate damage to the endothelial cells did not significantly affect the relative magnitude of the 10^{-5} M NA response as a percentage of the 40 mM K+-Krebs contraction, which was 109.2 ± 11.6% (n = 5), nor did it affect the magnitude of the relaxation induced by the alkaline shift in pH. However, damage to the endothelial cells did cause a significant further reduction in the acid-induced changes in NA response to 20 ± 3% (n = 5), P < 0.05 (Table 1). The baseline tension for pH 8.0 was frequently unstable, showing a gradual rise in tension with time accompanied by an increase in baseline noise. Only for concentrations of NA above 10^{-7} M was there obviously a contractile response to the NA.

pH Effect on Vessels Preactivated With KCl

The bar plot of Figure 4 shows the effect of pH changes on the K+-Krebs response in normal vessels (open bars). Note the small but significant reduction in response at pH 6.0 to 84 ± 6% (n = 25). At pH 8.0, there is a much larger reduction to 45 ± 5% (n = 24). Damage to endothelial cell function (hatched bars) did not significantly affect the pH-induced changes on K+-Krebs contraction (Table 1 and 2).

Summary

These acid- and alkaline-induced effects are summarized in the bar graph of Figure 5 for normal vessels only. Included in these data are the results at pH 7.0 for a smaller number of ring segments (n = 7). Control pH (filled bars), pH 6.0 (rising striped bars), pH 7.0 (open bars), and pH 8.0 (falling striped bars) are all compared for 10^{-5} M PGF_{2α} and NA, and 40 mM K+-Krebs. For PGF_{2α}, it is apparent that the response is relatively stable between pH 7.0 and 8.0, but it falls dramatically at pH 6.0. In contrast, both K+ and NA responses are clearly maximal at pH 7.4 and fall off in a graded fashion to both acidic and alkaline shifts.

FIGURE 4. Bar chart to compare the 40 mM K+-Krebs contractions in bath solutions of pH 7.4, 6.0, and 8.0 for vessels with (open bars) and without (hatched bars) a normal endothelium. Values are normalized to the response for pH 7.4 (n = 24–25).
Passive Tension
Passive tension was generally not sensitive to pH changes except on a few occasions at pH 8.0, where there was a small rise in passive tension.

Endothelial Cell Damage
Vessels precontracted with either NA or PGF$_{2\alpha}$ show graded relaxation responses to Ach. An example is shown in the raw data of Figure 6A for a vessel precontracted with $10^{-5}$ M PGF$_{2\alpha}$. The significant effect of deliberate endothelial cell damage on the relaxation response is shown in the average data of Figure 6B ($n = 6$). The relaxation response is plotted as a percentage reduction from the PGF$_{2\alpha}$ contraction, with closed circles representing the relaxation response with undamaged endothelial cells and open triangles after damage to endothelial cells. All points are statistically significantly different $P < 0.001$. There was no difference in the magnitude of the initial PGF$_{2\alpha}$ contraction with and without endothelial cells, indicating that they do not mediate the PGF$_{2\alpha}$ response.

DISCUSSION
Comparison of NA and PGF$_{2\alpha}$ Responses
NA is known to be a potent vasoconstrictive agent for the ophthalmic arteries of cat, dog, monkey, and man.$^{14-18}$ This is to be expected because of the presence of autonomic adrenergic innervation on this artery. These data demonstrate that PGF$_{2\alpha}$ is a more potent vasoconstrictor in this artery in the cat, producing approximately 50% more contraction for the same agonist concentration. In this respect, the data are the reverse of that reported for the monkey in which the NA response was considerable larger than that for PGF$_{2\alpha}$. Certainly, a species difference in the magnitude but not the sensitivity of the PGF$_{2\alpha}$ response in humans, dogs, and monkeys has already been demonstrated.$^{17,18}$ Bovine retinal arteries also contract when exposed to PGF$_{2\alpha}$, with similar response magnitudes of $1.37 \pm 0.13$ mN/mm$^2$ and $1.15 \pm 0.07$ mN/mm$^2$ compared with our value for the cat ophthalmic artery of $1.2$ mN/mm (Fig. 1). Moreover, the reported threshold concentration is similar ($10^{-6}$ M).$^{25}$ Prostaglandins such as PGF$_{2\alpha}$ are synthesized from arachidonic acid intracellularly and are released by local tissue or vascular mural cells causing vasoactivity. The specificity of many prostaglandin receptors already identified as active in biological systems is unclear, so a response to PGF$_{2\alpha}$ indicates but does not prove that local cells synthesize and release PGF$_{2\alpha}$ in the vicinity of the in vivo ophthalmic artery. The PGF$_{2\alpha}$ dose-response curves were not affected by damage to endothelial cell function, implying that the PGF$_{2\alpha}$ acts directly on the smooth muscle cells to produce contraction and does not rely on mediation through the endothelial cells. In this respect, NA and PGF$_{2\alpha}$ are similar.

Acidic Shift
Lowering the bathing pH solution to pH 7.0 has no effect on passive tension. However, at pH 7.0 both NA and K$^+$-Krebs responses are reduced, whereas the PGF$_{2\alpha}$ response remains unchanged. Further reduction in pH to values encountered during prolonged ischemia (6.0)$^{7,8}$ induces relaxation of the ophthalmic artery (an inhibition of the contraction response) independently of whether the vessel is contracted by PGF$_{2\alpha}$, NA, or K$^+$-Krebs, although the absolute magnitudes of this relaxation effect are significantly different for the three different vasoconstrictors ($P < 0.005$ in all cases), with the contractions falling to $23 \pm 4\%$ ($n = 23$), $40 \pm 4\%$ ($n = 23$), and $84 \pm 5\%$ ($n = 25$), respectively, of their values at pH 7.4. For PGF$_{2\alpha}$ and K$^+$-Krebs, this relaxation effect in acidic conditions is not modified by the loss of endothelial cell function. This means it cannot be attributed to a pH-stimulated release of nitric oxide or other relaxing factors such as PGI$_2$, the main metabolic product of arachidonic acid in isolated vascular tissue,$^{24,25}$ as has been observed for the canine jugular vein$^{26}$ and the rabbit aorta$^{27}$ with acidic shifts. This conclusion is further confirmed by the failure of prior treatment with indomethacin, an inhibitor of endogenous prostaglandin synthesis and release, to modify...
FIGURE 6. (A) Raw data to show a relaxation dose-response curve (10^{-10} M to 10^{-5} M) of ACh applied during a sustained precontraction with 10^{-5} M PGF_{2a} in a vessel with functioning endothelium. The vertical bar represents an isometric active tension of 0.5 mN/mm and the horizontal time bar, 4 minutes. (B) Normalized average dose-response curve to ACh before (closed circles) and after (open triangles) deliberate damage to endothelial cell function measured for vessels precontracted with 10^{-5} M PGF_{2a} (100%). All points statistically significantly different at P < 0.001.

the acidic relaxation response in our experiments. Endothelium-independent relaxation to PGF_{2a} at acidic pH was also observed by Toda et al., who used a variety of methods to induce acidity to differentiate between pH, pHi, hypercapnia, or altered bicarbonate concentration as stimuli for the induced relaxation in response to PGF_{2a}. They concluded that pH is the prime stimulus and that this is probably achieved through stimulation of the Na^+ pump in the smooth muscle cell.

The graded acid-induced relaxation that occurs for vessels contracted with K^+-Krebs was much smaller than for PGF_{2a}-activated vessels, implying that the mechanisms subserving the relaxation are different. This small relaxation effect (16% at pH 6.0) is largely confirmed by other studies on the rat cerebral artery, mesenteric artery, and aorta that found either no relaxation or a small relaxation effect in acidic conditions. The latter authors argue that the selectivity between receptor-mediated and K^+-Krebs activated vessels in the magnitude of the pH-induced relaxation suggests that modification of the contractile process itself is not implicated. They adduce some evidence that the acidic relaxation is due to increased Ca^{2+} sequestration for which pH must also fall. For the ophthalmociliary artery, the level of contractile activation cannot be the major factor determining acid relaxation because both NA and K^+-Krebs produce a similar activation magnitude but very different acidic relaxation effects. The graded acidic relaxation observed for NA-stimulated vessels was enhanced by endothelial cell damage (Table 1) at pH 6.0, which may mean that endothelial cells in the in vitro preparation do release vasoactive factors that reduce the relaxation effect of acidic shifts when NA receptors are active. There is evidence in other tissues too for a differential release of vasoactive autocoids from endothelial cells in response to variations in pH. Release of prostacyclin and thromboxane have both been shown to be affected by pH. However, the possibility that this is the "sparing" mechanism operative in this case for NA is unlikely because there was no evidence of release of vasoactive factors from endothelial cells in acidic conditions when the vessel was activated by PGF_{2a}. Other studies have confirmed a substantial acidic relaxation for vessels activated with NA, although the latter study shows a specificity for the α_2 rather than the α_1 adrenergic receptor, whereas the cat ophthalmociliary artery has been shown to possess only α_1 adrenergic receptors.

**Alkaline Shifts**

Alkaline shifts cause an endothelial-independent relaxation in both NA and K^+ activated segments of the ophthalmociliary artery. Previous studies have found both alkaline-induced relaxations for NA-activated vessels and contraction. In all these cases only pH was raised, leaving pH unchanged using the am-
This study has shown that the responses of some vasoconstrictive agents are selectively mediated by pH changes. For PGF$_{2\alpha}$, the response is stable over a wide range of pH, finally falling in extremely acidic conditions. In contrast, both NA and K$^+$ contractile activity is greatest at neutral pH, with both agents becoming less effective for acidic and alkaline conditions. What are the implications of these results for in vivo conditions? Normal tissue pHe changes in the ocular tissue caused by alterations in neural activity, such as changing from light to dark adaptation, are no larger than 0.2 to 0.4 log units of pHe.$^{19,37,38}$ Thus, one can conclude that these tissue metabolic changes, together with those due to systemic metabolic and respiratory acidosis and alkalosis, will be largely ineffective in altering either resting tension in this vessel or in altering the responses to NA and PGF$_{2\alpha}$. We have already shown that hypoxia only affects activation responses at low levels.$^{11}$ These facts lead to the conclusion that the ophthalmic blood flow and its distribution has to be studied from the moment-to-moment control of retinal and choroidal circulations closer to the site of tissue demand.

However, for truly ischemic conditions in which pH is much lower (6.0), the ophthalmic blood flow demonstrates considerable relaxation, reducing the efficacy of responses to NA or PGF$_{2\alpha}$. Such acid-induced relaxation has also been demonstrated over a much wider range of pH changes (5.0 to 7.4) in cat pial arterioles.$^{19}$ Understanding the control of retinal and choroidal blood flow is vital to improve our knowledge of retinal vascular disease, diagnosis, and possible therapeutic treatment. The studies performed in this laboratory have demonstrated that the ophthalmic blood flow is likely to be involved in responses to metabolic changes only in extreme conditions and that the dynamic moment-to-moment control of retinal and choroidal blood flow and its distribution has to be studied at more distal locations.

**Key Words**
ophthalmic artery, pH, noradrenaline, PGF$_{2\alpha}$, isolated vessel

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**References**

pH Effect on Ophthalmociliary Artery Response 1007