Retinal Arterial Tone Is Controlled by a Retinal-Derived Relaxing Factor

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Abstract—The present study provides evidence that retinal tissue may profoundly influence the retinal arterial smooth muscle cell tone by releasing an unknown retinal relaxing factor. Isolated bovine retinal arteries with and without adhering retinal tissue were mounted in a wire myograph for isometric tension recordings. The maximal contraction induced by prostaglandin F$_{2\alpha}$ was 0.95±0.7 mN (n=6) in the presence and 5.15±0.76 mN (n=6) in the absence of adhering retinal tissue. The contractions induced by U-46619, serotonin, and endothelin-1 were similarly blocked in the presence of retinal tissue. The K$^+$ 120 mmol/L-induced contraction was not significantly affected (2.8±0.7 mN, n=6, in the presence and 3.6±0.7 mN, n=6, in the absence of retinal tissue). Placing a piece of bovine retinal tissue in the proximity of a contracted (ie, with prostaglandin F$_{2\alpha}$) retinal artery induced a complete relaxation of the retinal vessel, suggesting the involvement of a diffusible chemical vasorelaxant. Also porcine, canine, and ovine retinal tissue completely relaxed the contracted (with prostaglandin F$_{2\alpha}$) bovine retinal artery. Other smooth muscle preparations, including rat mesenteric and renal arteries and rat main bronchi, also relaxed with the application of a piece of bovine retinal tissue. Incubation of bovine retinas in a Krebs-Ringer bicarbonate solution yielded a solution that relaxed isolated precontracted bovine retinal arteries, confirming the involvement of a diffusible chemical messenger. Hexane extraction, heating the solution to 70°C, or treatment with trypsin did not alter the relaxing properties of the incubation solution. The characteristics of the retinal relaxing factor do not correspond with those of nitric oxide, prostanooids, adenosine, acetylcholine, or any other of the known vasoactive neurotransmitters released from the retina. Our results suggest that retinal arterial tone is controlled by a diffusible, hydrophilic, and heat-stable relaxing factor that does not correspond with a known vasoactive molecule formed within the retina. (Circ Res. 1998;83:714-720.)

Key Words: retina ■ bovine ■ arteries ■ vasodilation ■ relaxation

Vascular smooth muscle tone is the result of myogenic tone modulated by circulating humoral factors and by neurotransmitters released from the vascular nerve endings. The original observations of Furchgott and Zawadzki1 make it obvious that vascular smooth muscle tone is also modulated by adjacent cells. In a study on isolated bovine retinal arteries, we accidentally observed that retinal arteries incompletely cleaned of retinal tissue contracted strikingly weaker in response to prostaglandin F$_{2\alpha}$ than retinal arteries without adhering retinal tissue. This suggests that retinal tissue might profoundly influence vascular smooth muscle tone in retinal arteries. The regulation of retinal circulation by local factors might be very important considering the absence in the retinal vessels of sympathetic innervation that predominantly controls the arterial tone in most other parts of the body. Indeed, sympathetic innervation of the central retina reaches only as far as the lamina cribrosa.2

A concept previously put forth is that surrounding retinal tissue might control retinal arterial tone. Donati et al3 suggested that nitric oxide (NO) released from the retina could control arterial tone. Prostaglandins derived from retinal tissue also have been proposed as mediators of retinal arterial dilation.4 Here we report that bovine retinal arterial tone is influenced by a diffusible relaxing factor from the retina that does not correspond, however, to the characteristics of NO or prostaglandins. We examined the potential role of the endothelium, noted some characteristics of the retinal relaxing factor (RRF), and compared the effect of the RRF with that of known vasoactive molecules formed within the retina. In addition, we investigated whether this RRF is released from retinas of other species and whether this factor relaxes nonretinal blood vessels and nonvascular smooth muscle preparations (eg, rat main bronchi). This was done to determine whether the RRF might be a more general relaxant agent.

Materials and Methods

Blood Vessels

Bovine eyes were obtained from the local slaughterhouse and transported to the laboratory in ice-cold Krebs-Ringer bicarbonate solution. Canine, porcine, and ovine eyes were enucleated from animals that were killed for other experiments. The extraocular muscles and connective tissues were dissected from the globe, the anterior segment and the vitreous were removed, and the eyecup was placed in cold Krebs-Ringer bicarbonate solution for further prepa-
ration. The retina was gently teased free from the choroid and cut loose at the optic nerve. The detached retina was then either placed in a cold Krebs-Ringer bicarbonate solution to isolate the retinal artery or incubated for 6 hours in a Warburg apparatus.

Under a dissecting microscope, arterial segments (209.9±2.5 μm, n=156) with surrounding retinal tissue were carefully excised from bovine retinas. Segments of the mesenteric arteries (328.6±12.1 μm, n=5), renal arteries (289.6±19.6 μm, n=5), and main bronchi (n=13) were isolated from male Wistar rats (300 g) that were killed by cervical dislocation. The animals were killed in accordance with a protocol that was approved by the local ethics committee.

**Tension Measurements**

The vessels were moved to an automated dual myograph (model 500 A, JP Trading, Aarhus, Denmark) containing 10 mL Krebs-Ringer bicarbonate solution. Two stainless steel wires were guided through the lumen of the vessels. One wire was fixed to a force displacement transducer, and the other was connected to a micrometer. The retinal tissue remained attached to the retinal arteries or, when necessary, was removed carefully after the first wire was fixed.

The segments were allowed to equilibrate for half an hour in the Krebs-Ringer bicarbonate solution bubbled with 95% O₂/5% CO₂ and heated to 37°C. Then the passive wall tension–internal circumference characteristics of the vessels were determined. On the basis of this relationship, the circumference was set to a normalized internal circumference. In the experiments on rat main bronchi, the preparations were stretched to 5 mN.

After the normalization procedure, the vessels were repeatedly activated with K⁺ 120 mmol/L. Maximal contractility was assessed by stimulating the arteries simultaneously with K⁺ 120 mmol/L, prostaglandin F₂α (PGF), 30 μmol/L, and serotonin 10 μmol/L. The main bronchi were repeatedly contracted with K⁺ 120 mmol/L and carbacbol 1 mmol/L.

**Removal of the Endothelium**

For this procedure, the arteries were unstretched in the myograph. An L-shaped micropipette was positioned at the proximal end of the vessel, and 95% O₂/5% CO₂ was bubbled through the lumen for 2 minutes. Thereafter, the wires were reset to their original positions, and the vessel was allowed to reequilibrate for half an hour. The absence of endothelium was tested by the lack of acetylcholine-induced (10 μmol/L) relaxation.⁷

**Incubation**

Detached retinas were incubated in pairs (weight, 1650 mg each) in 20 mL oxygenated Krebs-Ringer bicarbonate solution heated at 37°C for 6 hours using a Warburg apparatus. After incubation, the retinas were removed from the flask, and the remaining solution was centrifuged at 3000 rpm for 10 minutes. After centrifugation, the supernatant was frozen and stored. When sufficient amounts were collected, the incubation fluid of different retinas was centrifuged at 3000 rpm for 10 minutes. In the initial series of experiments, the solution was extracted 3 times with hexane [Figure 4a]. After centrifugation, the hexane fraction was discarded. This procedure allowed a partial purification of the solution to occur. The hexane-extracted solution was then directly tested or exposed to heating to 70°C for 1 hour or treatment with trypsin (1 μg/mL) during 2 hours at 37°C). A Krebs-Ringer bicarbonate solution incubated without retinas but treated in the same manner as the solution incubated with retinas served as a control solution in these experiments.

**Bioassay**

The arteries were mounted, normalized, and activated as described above. After these procedures, the vessels were superfused at a rate of 0.25 mL/min with warmed (37°C) and bubbled (95% O₂/5% CO₂) test solutions.

**Figure 1.** Typical experiment in which the contractions of an isolated bovine retinal artery with adhering retinal tissue (top tracing) are compared with the contractions of a retinal artery that was carefully dissected free of surrounding tissue (bottom tracing). The vessels are first maximally contracted with K⁺ 120 mmol/L to which serotonin (SER), 10 μmol/L and prostaglandin F₂α (PGF), 30 μmol/L are added. During this procedure, both vessels show a very similar reactivity. However, in response to increasing concentrations of prostaglandin F₂α (given as the final-log–molar concentration in the organ bath), the retinal artery with adhering retinal tissue shows only a weak contraction compared with the cleaned preparation. Vertical scale shows the active force in mN; horizontal scale shows time; and W indicates a wash with Krebs-Ringer bicarbonate solution.

**Drugs**

The experiments were performed using a Krebs-Ringer bicarbonate solution of the following composition (mmol/L): NaCl 135, KCl 5, NaHCO₃ 20, glucose 10, CaCl₂ 2.5, MgSO₄ 7, H₂O 1.3, KH₂PO₄, 1.2, and EDTA 0.026. A Krebs-Ringer bicarbonate solution containing 120 mmol/L K⁺ was prepared by equimolar replacement of NaCl with KCl.

U-46619, serotonin (5-hydroxytryptamine), acetylcholine chloride, indomethacin, L-NA, endothelin-1, L-glutamic acid, adenosine, 8-phénylthéophylline, dopamine, melatonin, and tetrodotoxin were obtained from Sigma Chemical Co; prostaglandin F₂α, dinoprost trometamolum (dinoprost trometamolum, Dinolytic) from Upjohn (Puurs, Belgium); and γ-aminobutyric acid (GABA) from Interlaboratoire (Wezenbeek, Belgium). Methylene blue was purchased from Merck (Darmstadt, Germany). Trypsin 10x [1:250] was obtained from Gibco (Merelbeke, Belgium). Stock solutions were made in water, except for the indomethacin, which was dissolved in ethanol, and 8-phénylthéophylline, which was dissolved in DMSO.

**Results**

**Influence of Retinal Tissue on Contractions Induced by Different Agonists**

In the first series of experiments, the contractile responses of arteries that were dissected free from all retinal tissue (control preparations) and arteries to which a piece of retinal tissue remained attached were compared. An original tracing of such an experiment is represented in Figure 1. In response to increasing concentrations of prostaglandin F₂α (0.1 to 30 μmol/L), the retinal artery with adhering retinal tissue showed only a weak contraction compared with the preparation without retinal tissue; however, both preparations (with and without retinal tissue) contracted in a similar way in response to a standard activating solution containing K⁺.
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120 mmol/L, serotonin 10 μmol/L, and prostaglandin F2α 30 μmol/L.

Contraction induced by serotonin (1 mmol/L to 10 μmol/L), endothelin-1 (0.1 pmol/L to 0.1 μmol/L), and the thromboxane A2 mimetic, U-46619 (1 mmol/L to 1 μmol/L) (Figure 2) were also less pronounced in the presence of retinal tissue. Retinal tissue does not appear to mechanically impair the contractions because the contractile response to K+ 120 mmol/L was similar in preparations with (2.8±0.7 mN, n=6) and without (3.6±0.7 mN, n=6) adhering retinal tissue.

Effect of L-NA and Indomethacin

The NO-synthase inhibitor L-NA and the cyclooxygenase blocker indomethacin were used to exclude the potential influence of NO or cyclooxygenase metabolites synthesized by the retinal tissue. Concentration-response curves for prostaglandin F2α (10 nmol/L to 30 μmol/L) were constructed in the presence or absence of L-NA (0.1 mmol/L, incubated for 10 minutes) or indomethacin (10 μmol/L, incubated for 20 minutes) on retinal arteries with and without adhering retinal tissue.

Indomethacin did not significantly alter the inhibitory influence of the adhering retinal tissue. The maximal contraction induced by prostaglandin F2α on the retinal artery with adhering retinal tissue was 0.22±0.09 mN (n=6) before the addition of indomethacin and 0.30±0.16 mN after the addition of indomethacin. L-NA enhanced (P<0.025) the maximum contraction induced by prostaglandin F2α of the preparation with adhering retinal tissue (1.60±0.34 mN, n=6); however, L-NA also significantly (P<0.05) enhanced the sensitivity of the preparation without adhering retinal tissue (log EC50, −4.841±0.301, n=6 before incubation with L-NA and −5.360±0.161 after incubation with L-NA).

**Effect of Tetrodotoxin**

Concentration-response curves for prostaglandin F2α (10 nmol/L to 30 μmol/L) were constructed in the absence or presence of tetrodotoxin ([TTX] 10 μmol/L) on retinal arteries with and without adhering retinal tissue. TTX had no significant influence on the maximum contractions induced by prostaglandin F2α on either preparation. Prostaglandin-induced contractions of the arteries without adhering retinal tissue (n=3) were 7±0.9 mN in the absence of TTX and 5.9±1.2 mN in the presence of TTX. The maximal contractions of the preparations with adhering retinal tissue (n=5) were 0.1±0.1 mN and 0.3±0.2 mN, respectively, in the absence and presence of TTX.

**Effect of Detached Retinal Tissue of Different Species on Contractions Induced by Prostaglandin F2α and High Potassium**

This series of experiments was performed to find out whether a diffusible chemical messenger was responsible for the inhibitory effect of the retinal tissue. A ring segment of the retinal artery that was carefully cleaned of all retinal tissue was mounted for isometric tension recording, contracted with prostaglandin F2α (30 μmol/L), and then covered with a piece of retinal tissue (±230 mm2) (schematic representation, Figure 3). This elicited a complete and stable relaxation (100±3.3% relaxation, n=8) of the vessel that rapidly recovered when the retina was removed (Figure 4a). The same arteries contracted with K+ 120 mmol/L relaxed significantly (P<0.001) weaker with application of the retinal tissue (46.6±6.0% relaxation, n=8) (Figure 4a), confirming the initial observations that the presence of retinal tissue had only a moderate influence on K+ 120 mmol/L–induced contractions. A similar-sized piece of choroidal tissue (Figure 4b) was incapable of inducing such a pronounced relaxation (13±2.7% relaxation, n=7) of the retinal artery contracted with prostaglandin F2α (30 μmol/L).

In additional experiments, we investigated whether application of canine (n=2), porcine (n=4), or ovine (n=2) retinas would relax a bovine retinal artery contracted with prostaglandin F2α (30 μmol/L). All of the retinas from these different species completely relaxed bovine retinal arteries (Figure 4b).
Role of the Endothelium

During this series of experiments, we investigated whether the presence of the endothelium was required for the retina-induced relaxations. Retinal arteries without retinal tissue were first contracted with prostaglandin F_{2a} (30 μmol/L). When the contraction reached a steady state, the retinal artery was covered with a piece of retinal tissue (±230 mm²), resulting in pronounced relaxation. This procedure was repeated after the endothelium was removed and after the absence of functional endothelium was confirmed by the lack of relaxation induced by acetylcholine (10 μmol/L). Removal of the endothelium did not alter the relaxations induced by the retinal tissue. The relaxation of the arteries before removal of the endothelium was 99.3±2.8% and after removal of the endothelium was 98.9±0.9% (n=6).

Influence of Methylene Blue on the Relaxations Induced by the Application of a Piece of Retinal Tissue

Methylene blue, a blocker of guanylyl cyclase, was used to exclude the involvement of cGMP-dependent mechanisms. Isolated bovine retinal arteries without adhering retinal tissue were contracted with prostaglandin F_{2a} (30 μmol/L), after which the vessels were covered with a piece of bovine retinal tissue. This resulted in a complete relaxation (100%, n=8) of the bovine retinal arteries. The same procedure was repeated 20 minutes after methylene blue (5 μmol/L) was added to the organ bath. Methylene blue induced a marked contraction (1.4±0.4 mN, n=8) of the retinal arteries. The relaxation induced by the application of the retinal tissue, however, was not affected. The application of the retinal tissue still induced a complete relaxation after incubation with methylene blue.

Influence of Retinal Tissue on Rat Renal and Mesenteric Arteries and on Rat Main Bronchi

Our investigation included whether other arteries and nonvascular smooth muscle preparations would relax in the presence of retinal tissue. Therefore, rat mesenteric and renal arteries were contracted with prostaglandin F_{2a} (30 μmol/L), and rat main bronchi were contracted with carbachol (0.1 mmol/L). Application of a piece of retinal tissue (±230 mm²) relaxed both the rat mesenteric (40.7±11.2% relaxation, n=5) and renal (68.0±13.3% relaxation, n=5) arteries (Figure 4c). Also, the rat main bronchi relaxed when they were brought in close contact with a piece of bovine retinal tissue (52.8±10.6% relaxation, n=7).

Bioassay

Having established that the relaxing effect of the retinal tissue was most likely caused by a relaxing factor released by the retina, a bioassay setup was developed to analyze some characteristics of this RRF. In this technique, an isolated bovine retinal artery (detector preparation) was mounted for isometric tension recording. The artery was continuously superfused with warmed and bubbled Krebs-Ringer bicarbonate solution containing prostaglandin F_{2a} (30 μmol/L) to precontract the detector preparations. Changing the superfusion from a solution without RRF to a solution with RRF (obtained by incubation for 6 hours with bovine retina) elicited a relaxation (32.5±13.2%, n=4) of the detector preparation. The relaxing influence of the RRF-containing solution was not significantly changed after extraction with hexane (10 mL solution extracted 3 times with 10 mL hexane/38.5±8.2% relaxation, n=4, Figure 5a). Heating the solution at 70°C for 1 hour did not alter the relaxing effect (33.2±6% relaxation, n=4, Figure 5b). Treatment with trypsin (1 μg/mL, incubated for 2 hours at 37°C) had no influence although the same treatment abolished the contraction induced by an endothelin-1–containing (40 nmol/L) solution (Figure 5c). The relaxation induced by the RRF-containing solution before treatment with trypsin (24.9±10.5%) compared well with the relaxation after treatment (24.5±8.7%, n=3).

pH Measurements of the Medium and the RRF-Containing Solution

In this series of experiments, the pH of the medium and the pH of the incubation solution, which was obtained after 6...
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Figure 5. Tracings of 3 different bioassay experiments. The detector preparation in all 3 experiments is an isolated bovine retinal artery contracted with prostaglandin F2α (30 μmol/L). a. Relaxation induced by an RRF-containing solution before (RRF) and after hexane extraction (RRF&H). A control solution extracted with hexane (C&H) does not affect the contraction induced by prostaglandin F2α. b. Relaxation induced by an RRF-containing solution before (RRF) and after exposure to 70°C during 1 hour (RRF&70°C). A control solution exposed to 70°C (C&70°C) does not induce a relaxation. c. Contracture induced by endothelin-1 (ET, 40 nmol/L) and the relaxation induced by RRF. Incubation with trypsin 1 μg/mL (ET&T) at 37°C during 2 hours completely abolishes the endothelin-1 contraction. The relaxing effect of the RRF-containing solution persists after trypsin treatment (RRF&T). Trypsin (T) alone does not affect the contraction induced by prostaglandin F2α. W indicates a wash with Krebs-Ringer bicarbonate solution.

Correcting the pH of the RRF-containing solution did not significantly change the relaxing properties of the RRF-containing solution. Sodium bicarbonate was added to the RRF-containing solution to correct pH to normal values (pH=7.31±0.015, n=5). The relaxation induced by the pH-corrected solution (24.5±4.8%, n=5) was not significantly different from the relaxation induced by the pH-uncorrected solution (28.5±4.9%, n=5).

Influence of Different Retinal Neurotransmitters on the Retinal Arterial Tone

The retina releases various neurotransmitters that might influence the retinal arterial tone. In this series of experiments, the effect of various known retinal neurotransmitters on retinal arteries cleaned from all surrounding retinal tissue was investigated. High concentrations of glutamate (1 mmol/L, n=6), glycine (1 mmol/L, n=6), and GABA (0.1 mmol/L, n=2) had no influence on the retinal arteries contracted with prostaglandin F2α (30 μmol/L). Increasing concentrations of melatonin (10 nmol to 10 μmol/L, n=2) and dopamine (10 mmol to 0.1 mmol/L, n=6) also did not relax the retinal arteries contracted with prostaglandin F2α (30 μmol/L). Adenosine (10 μmol/L to 0.1 mmol/L, n=6) induced a concentration-dependent relaxation of the retinal artery contracted with prostaglandin F2α (30 μmol/L). At a concentration of 0.1 mmol/L, adenosine induced a 52.7±12.9% (n=7) relaxation. In additional experiments, the effect of adenosine (10 μmol/L to 0.1 mmol/L) was tested on rat main bronchi contracted with carbachol (0.1 mmol/L). Adenosine did not relax the rat main bronchi and at high concentrations even showed a tendency to increase the contraction induced by carbachol (an increase of 18.7±3.4%, n=7).

Effect of 8-Phenyltheophylline on the Relaxation Induced by the Retinal Tissue

To further exclude the involvement of adenosine in the relaxing effect of the retinal tissue, 8-phenyltheophylline (10 μmol/L), an aspecific adenosine receptor blocker, was used. 8-phenyltheophylline (10 μmol/L) significantly (P<0.05) blocked the adenosine-induced (10 μmol/L) relaxation of the retinal artery contracted with prostaglandin F2α (30 μmol/L). This is a 4.2±1% relaxation in the presence of 8-phenyltheophylline compared with a 34.5±11.6% relaxation in the absence of 8-phenyltheophylline (n=6). The presence of 8-phenyltheophylline, however, did not alter significantly the relaxations induced by the application of a piece of the retinal tissue.

Discussion

The main finding of the present study is that retinal tissue exerts a marked inhibitory influence on the contractile tone of isolated retinal arteries. These observations cannot be ascribed to mechanical hindrance, because the contractions induced by K+ 120 mmol/L or by the standard activating solution (containing K+ 120 mmol/L, prostaglandin F2α, and serotonin) are not significantly affected by the presence of retinal tissue.

The adhering retinal tissue probably influences the vascular smooth muscle cell through a diffusable chemical messen-
The initial demonstration of the involvement of RRF required the apposition of detached retinal tissue to a detector preparation. The detector preparation in the initial experiments was a bovine retinal artery cleaned of all retinal tissue and contracted with prostaglandin F\(_{2\alpha}\). In later experiments, the bovine retinal artery was replaced by other arteries (eg, rat mesenteric and renal arteries) and another smooth muscle preparation (rat main bronchi). These different preparations also relaxed on application of retinal tissue, which suggests that RRF can be considered a more general smooth muscle relaxant. Nevertheless, the retinal artery seemed to be the most responsive of all preparations tested.

Besides changing the detector preparation, the source of the RRF was also changed. Retinas of pigs, dogs, and sheep also seemed to release a relaxing factor, because application of these tissues also fully relaxed the bovine retinal arteries contracted with prostaglandin F\(_{2\alpha}\). No such relaxation was seen when a similar-sized piece of bovine choroidal tissue was placed in the proximity of the artery, which excluded the involvement of mechanical disturbances in this kind of experiment.

Retinal arteries contracted with K\(^+\) 120 mmol/L relaxed significantly less on application of retinal tissue. The presence of retinal tissue had only a moderate influence on K\(^+\) 120 mmol/L–induced contractions. The moderate relaxation of the artery contracted with K\(^+\) 120 mmol/L might be explained by a decreased release of the RRF from the depolarized retina or a decreased sensitivity of the depolarized arterial smooth muscle cells to the RRF.

The existence of this tentative RRF was confirmed by incubating bovine retinas in Krebs-Ringer bicarbonate solution and testing the effect of the incubation solution on isolated and cleaned retinal arteries. This solution elicited a relaxation of the retinal artery. The relaxing influence of the RRF-containing solution was not changed after extraction with hexane. Heating the solution to 70°C for 1 hour did not alter the relaxing effect. Treatment with trypsin also had no influence although the same treatment abolished the contraction induced by an endothelin-1–containing solution. The relaxations induced by the RRF-containing solution could not be attributed to changes in pH, because the pH changes alone were not sufficient to influence the retinal arterial tone. These data suggest that RRF is hydrophilic, thermostable, and is not a polypeptide or protein.

That the retinal arterial tone may be controlled by mediators released from the surrounding retinal tissue has already been suggested. Both NO\(^1\) and prostaglandin E\(_2\)\(^4\) have been proposed as possible factors. However, both can be excluded from being the RRF.

NO has a very short half-life and would be destroyed after 1 hour at 70°C. In addition, NO induces only a moderate relaxation of the bovine retinal artery.\(^3\) L-NA (0.1 mmol/L), a NO synthesis blocker, was unable to abolish the inhibitory influence of the adhering retinal tissue although its presence enhanced the prostaglandin F\(_{2\alpha}\)–induced contractions. We think that this is caused by an inhibition of the basal release of NO from the endothelium, because L-NA also significantly enhanced the sensitivity of the preparation without the adhering retinal tissue. Spontaneous release of NO from the endothelium is also suggested by the contraction induced by methylene blue,\(^6\) a blocker of guanylyl cyclase. Methylene blue had no effect on the relaxations induced by the application of a piece of retinal tissue on a bovine retinal artery contracted with prostaglandin F\(_{2\alpha}\). This is another argument against the involvement of NO in the retina-induced relaxations.

Indomethacin (10 \(\mu\)mol/L), a cyclooxygenase inhibitor, did not alter the concentration-response curves to prostaglandin F\(_{2\alpha}\) when used on preparations with adhering retinal tissue, which argues against the RRF being prostaglandin E\(_1\) or another cyclooxygenase product.

Because the RRF did not correspond with already proposed relaxing factors from the retina, the potential role of other substances formed by the retina was investigated. The neural retina consists of 6 types of neurons: photoreceptor, horizontal, bipolar, amacrine, interplexiform, and ganglion cells.\(^10\) Müller cells and retinal pigment epithelium are the two principal nonneuronal cells of the retina.\(^11\) Astrocytes are present in the nerve-fiber layer but are absent from the deeper retinal laminae. Each of these cells releases various molecules, of which some have vasoactive properties and thus are potential candidates for being the RRF. The neurotransmitter released by rod-and-cone photoreceptor cells is generally thought to be glutamate.\(^12\) Glutamate also appears to be the major neurotransmitter of the cone bipolar cells and the ganglion cells.\(^13\) Glycine and GABA are also present in various retinal neuronal cells.\(^12\) However, glutamate, glycine, or GABA does not induce relaxation of isolated retinal arteries contracted with prostaglandin F\(_{2\alpha}\). Dopamine is the principal catecholamine transmitter of vertebrate retina.\(^13\) Dopamine, however, is also unable to induce a relaxation of the bovine retinal artery contracted with prostaglandin F\(_{2\alpha}\). Melatonin, which is released by the retina, is also incapable of relaxing these arteries. From the various peptides that are released, calcitonin gene-related peptide (CGRP) was the most likely candidate. Prieto et al\(^14\) reported that CGRP invariably induced a slow-acting but potent relaxation of isolated bovine retinal arteries contracted with prostaglandin F\(_{2\alpha}\). As mentioned above, treatment of the incubation solution with trypsin did not alter the relaxation induced by the RRF, which not only makes the involvement of CGRP unlikely but also excludes other peptides, such as vasoactive intestinal peptide, substance P, and somatostatin. Acetylcholine is a neurotransmitter of the starburst amacrine cells.\(^12\) Acetylcholine induces a moderate endothelium-dependent relaxation of the isolated retinal artery.\(^7,9\) Because the relaxation induced by the retinal tissue occurs independently of the endothelium, acetylcholine and other endothelium-dependent vasodilators, (eg, histamine)\(^15\) are unlikely to be the RRF.

Adenosine is a stable, hydrophilic molecule and is capable of relaxing the isolated retinal artery contracted with prostaglandin F\(_{2\alpha}\). However, considerably high concentrations (10 \(\mu\)mol/L to 100 \(\mu\)mol/L) are needed to induce a pronounced relaxation of the retinal artery. The presence of 8-phenyltheophylline (10 \(\mu\)mol/L), an aspecific adenosine receptor blocker, did not alter the relaxations induced by application of the retinal tissue. Adenosine is unable to relax isolated rat main bronchi contracted with carbachol, whereas
the retinal tissue induces a relaxation of this same preparation. Moreover, adenosine even slightly contracts isolated rat main bronchi. These data thus exclude adenosine from being the RRF.

The identity of the RRF thus remains to be established. Also, the cell type releasing RRF is as yet unknown. The presence of tetrodotoxin (10 μmol/L) did not significantly alter the inhibitory effect of the retinal tissue, which suggests that glial rather than neuronal cells release this factor. Also, the conditions of release of this elusive factor and the way RRF relaxes smooth muscle cells remain to be determined. The RRF seems to be released continuously from the isolated retina, because the inhibitory influence exerted by the retinal tissue does not diminish during prolonged experiments.

Continuously released RRF might have a physiological role in regulating retinal blood flow, because atrophy of the retina decreases the retinal vessel diameter. Retinitis pigmentosa, panretinal photocoagulation, and descending optic atrophy are pathologies with very different origins but with two characteristics in common: namely, retinal cell loss and attenuated retinal vessels (ie, decreased diameters). Attenuation of the retinal vessels is attributed to vascular remodeling caused by decreased metabolic demand, which is secondary to the loss of retinal cells.16 Vascular remodeling arises from sustained changes of blood flow.16 Changes in retinal blood flow should be seen before vascular remodeling. In early stages of retinitis pigmentosa, the changes in retinal blood flow can be detected before ophthalmoscopic signs and atrophy of the retinal vessels.17 Destruction of the outer retina may allow choroidal oxygen to diffuse to the inner retina, resulting in increased oxygen levels and autoregulatory vasoconstriction.18,19 This hypothesis may explain the narrow vessels in retinitis pigmentosa and panretinal photocoagulation. In descending optic atrophy, the primary lesion is located in the optic nerve and affects the inner retina, which makes this hypothesis, at least in this disease, less probable. Furthermore, the vessels in retinitis pigmentosa show a normal regulatory response to hyperoxia, suggesting that there is no hyperoxia in the inner retina of patients with retinitis pigmentosa.19 The mechanisms responsible for the changes in blood flow are obscure but might be explained by a lack of RRF. However, the physiological role of RRF in the retinal circulation can be established only after the RRF is identified and selective antagonists become available.

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