Sildenafil (Viagra) Evokes Retinal Arteriolar Dilation: Dual Pathways via NOS Activation and Phosphodiesterase Inhibition

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PURPOSE. Sildenafil (Viagra; Pfizer, New York, NY), a selective phosphodiesterase type-5 (PDE5) inhibitor, is widely used to treat impotence by improving penile blood flow via elevation of cGMP. However, its effect on ocular circulation is controversial and whether retinal arterioles are responsive to this drug remains unclear. In this study, the direct reaction of retinal arterioles to sildenafil was examined and the signaling pathway underlying this vasomotor activity was probed.

METHODS. Retinal arterioles from porcine eyes were isolated, cannulated, and pressurized without flow. Diameter changes in response to sildenafil were recorded using videomicroscopic techniques.

RESULTS. Retinal arterioles (67 ± 2 μm) dilated dose dependently to sildenafil (1 ng/mL to 1 μg/mL). This dilation was inhibited by the nitric oxide (NO) synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME), the guanylyl cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), the extracellular signal-regulated kinase (ERK) pathway inhibitor PD98059, the nonselective potassium channel blocker tetraethylammonium (TEA), and the selective adenosine triphosphate (ATP)-sensitive potassium (KATP) channel blocker glibenclamide. The vasodilation elicited by the NO donor S-nitroso-N-acetylpenicillamine (SNAP) was inhibited by ODQ and TEA but was insensitive to PD98059. In the presence of L-NAME, the addition of SNAP (1 μM) produced modest vasodilation and the inhibited sildenafil response was subsequently restored. The restored dilation was insensitive to PD98059 but was blocked by TEA.

CONCLUSIONS. Activation of NO synthase, through ERK signaling, leading to NO production and subsequent guanylyl cyclase activation and KATP channel opening is the major vasodilatory pathway for sildenafil in retinal arterioles. Moreover, the elevated cGMP, from endogenous or exogenous NO, plays a permissive role for sildenafil to exert vasodilation through inhibition of the PDE5 pathway independent of ERK signaling.

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Purpose: Sildenafil (Viagra; Pfizer, New York, NY) is the first commercially available selective inhibitor of phosphodiesterase-5 (PDE5) to be widely used for the treatment of erectile dysfunction. The inhibition of PDE5 can enhance the relaxation effect of nitric oxide (NO) on the smooth muscle lining of both the arteries and the sinusoidal spaces of the corpora cavernosa by reducing cyclic guanosine monophosphate (cGMP) degradation and thus increase arterial blood flow into penile sinusoids for erection. However, visual disturbances (e.g., changes in color and light perception, alterations in retinal electrophysiology, conjunctival hyperemia, and blurred vision)1–3 and nonarteritic anterior ischemic optic neuropathy4,5 have been reported in association with the use of sildenafil. Although it has been suggested that some of these symptoms may be related to the additional inhibition of PDE6 by sildenafil in photoreceptor cells,1–3 it remains unclear whether alteration of the ocular circulation, directly or indirectly through local and/or systemic blood pressure changes, by sildenafil is involved.4

In the past 5 years, several investigators have studied the effect of sildenafil on the ocular circulation in humans. An increase in pulsatile ocular–choroidal blood flow and retinal microcirculatory flow velocity by sildenafil has been reported in healthy human subjects.6,7 However, the ineffectiveness of sildenafil on optic nerve rim and choroidal circulation was also observed in healthy human subjects. In a small cohort of healthy persons or patients with erectile dysfunction,11 sildenafil had no effect on blood velocity in the central retinal artery but caused a significant increase in mean velocity in the ophthalmic artery.10,11 In addition to these inconsistent findings, direct measurements of retinal vessel diameter in healthy human subjects also yield conflicting results. Dilation of the superior temporal artery12 and vein12,13 in the human retina in response to sildenafil was reported by some investigators, but others found the absence of vasomotor action of sildenafil.14 Although these apparent discrepancies may be a result of different methods, techniques, and/or sensitivities in measuring ocular blood flow and vascular diameter, those observations may also have been confounded by the activation of neural, humoral, local, and/or autoregulatory flow regulation mechanisms secondary to sildenafil administration. Therefore, it remains unclear whether sildenafil exhibits direct vasomotor action in the ocular circulation. To investigate, we used in vitro isolated vessel techniques to determine directly whether sildenafil exerts vasodilatory action in retinal arterioles and to elucidate the underlying signaling pathways responsible for this vasomotor activity.

Methods

Animal Preparation

All animal procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Scott & White Institutional Animal Care and
Use Committee. Pigs (8–12 weeks old of either sex; 7–10 kg) purchased from Barfield Farms (Rogers, TX) were sedated with telentamine-zolazepam (4.4 mg/kg, IM; Telazol, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (2.2 mg/kg, IM), anesthetized with pentobarbital sodium (30 mg/kg, IV), intubated, and ventilated with room air. Heparin (1000 U/kg) was administered into the marginal ear vein to prevent clotting, and the eyes were enucleated and immediately placed in a moist chamber on ice.

**Isolation and Cannulation of Microvessels**

The preparation of isolated retinal arterioles has been described in our previous studies. In brief, the anterior segment and vitreous body from the porcine eye were removed carefully under a dissection microscope. The eye cup was placed in a cooled dissection chamber (8°C) containing a physiological salt solution (PSS; in mM: NaCl 145.0, KCl 4.7, CaCl2 2.0, MgSO4 1.17, NaH2PO4 1.2, glucose 5.0, pyruvate 2.0, EDTA 0.02, and MOPS 3.0) with 1% albumin (USB, Cleveland, OH). Second-order retinal arterioles (40–50 μm in internal diameter in situ and 0.6–1.0 mm in length without branches) in the temporal region of the retina were carefully dissected with Vannas spring scissors and a pair of Dumont microdissection forceps (Fine Science Tools, Foster City, CA) with the aid of a stereo microscope (model SZX12; Olympus, Melville, NY). An arteriole was then transferred for cannulation to a Lucite vessel chamber containing PSS-albumin solution. Both ends of the arteriole were cannulated with glass micropipettes (tip outer diameter of 30–40 μm) filled with PSS-albumin solution, and the outside of the arteriole was securely tied to the pipettes with 110 nylon sutures (Alcon, Fort Worth, TX). After cannulation, the vessel and pipettes were transferred to the stage of an inverted microscope (model CKX41; Olympus) coupled to a video camera (Sony DXC-190; Labtek, Campbell, CA) and video micrometer (Cardiovascular Research Institute, Texas A&M Health Science Center, College Station, TX) for continuous measurement of the internal diameter. The arterioles were pressurized to 55 cmH2O without flow by two independent reservoirs based on pressure ranges that have been documented in retinal arterioles in vivo and in the isolated, perfused retinal microcirculation. Preparations with leaks were excluded from further study.

**Experimental Protocols**

Cannulated arterioles were bathed in PSS-albumin at 36 to 37°C to allow development of basal tone. After a stable basal tone was achieved (~30–40 minutes), the concentration-dependent response to sildenafil (1 ng/mL to 1 μg/mL) was established. In some vessels, the response was re-examined after 30 to 60 minutes, to confirm the reproducibility. To determine the possible signaling pathways involved in the retinal arteriolar response to sildenafil, the following series of experiments were performed. The role of nitric oxide synthase (NOS) in mediating the vascular response was assessed in the presence of a known effective concentration of the specific inhibitor L-NAME, 10 μM. The involvement of soluble guanylyl cyclase, extracellular signal-regulated kinase (ERK), and potassium channel activation was assessed by the specific inhibitors 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 0.1 μM) and nonselective potassium channel inhibitor tetraethylammonium (TEA, 20 mM) or the selective adenosine triphosphate (ATP)-sensitive potassium (KATP) channel inhibitor glibenclamide (5 μM). To determine whether NO-induced vasodilation is mediated by the activation of ERK and guanylyl cyclase and the opening of potassium channels, the vascular response to the NO donor SNAP was examined in the presence of PD98059, ODQ, and TEA, respectively. In a separate series of experiments, the vascular response to sildenafil was examined in the presence of L-NAME after adding SNAP (1 μM). The effects of PD98059 and TEA on sildenafil-induced vasodilation were subsequently examined. All drugs were administered extraluminally, and each antagonist was incubated with the vessels for at least 30 minutes.

**Sildenafil-Induced Dilation of Retinal Arterioles**

Sildenafil citrate was obtained from Pfizer. Other drugs were obtained from Sigma-Aldrich (St. Louis, MO) except where specifically stated otherwise. L-NAME, TEA, and sodium nitroprusside were dissolved in PSS, and SNAP was dissolved in water. Sildenafil, ODQ, and PD98059 were dissolved in ethanol and subsequently diluted in PSS. Glibenclamide was dissolved in dimethyl sulfoxide (DMSO). Vehicle control studies were performed in parallel with drug administration, and the highest concentration of organic solvents (0.1% ethanol and 0.03% DMSO) had no effect on the basal tone or arteriolar function, as reported in our previous studies.

**Data Analysis**

The changes in inner diameters of arterioles in response to agonists in the absence and presence of various inhibitors were recorded throughout the experiments. At the end of each experiment, the vessel was relaxed with 0.1 mM sodium nitroprusside in calcium-free PSS containing EDTA (1 mM) to obtain its maximal diameter. The dilations of retinal arterioles to agonists were normalized to their maximal vasodilatory capacity and expressed as a percentage of maximal dilation, based on the following formula: (response diameter − baseline diameter)/(maximal diameter − baseline diameter) × 100 = % maximal dilation. Data are reported as the mean ± SEM, and n represents the number of vessels studied. Statistical comparisons were performed by two-way ANOVA followed by the Bonferroni multiple-range test. The changes in resting tone by pharmacologic antagonists were analyzed by paired Student’s t test. P < 0.05 was considered significant. Taking into account the observed variability in responses (SD) when applying ANOVA, the sample size for each protocol in the present study is sufficient to detect a 5% change in dilation with the power of at least 0.80 when α = 0.05.

**Results**

**Dilation of Retinal Arterioles to Sildenafil**

In this study, all vessels developed a similar level of basal tone (constricted to 69% ± 1% of their maximal diameter) at 36 to 37°C bath temperature with 55 cmH2O transluminal pressure. The average resting and maximal diameters of the vessels were 67 ± 2 and 95 ± 2 μm, respectively. Sildenafil produced dose-dependent (1 ng/mL to 1 μg/mL) dilation of retinal arterioles, and the dilation was completely developed within 2 to 3 minutes at each concentration. The highest concentration of sildenafil (1 μg/mL) elicited 31% ± 2% of maximum dilation (Fig. 1). To avoid the confounding effects from the high concentration of solvent (i.e., ethanol), the concentration of sildenafil higher than 1 μg/mL was not examined. The sildenafil-elicited dilation was reproducible and did not deteriorate with repetitive applications (Fig. 1).

**Role of NOS and Guanylyl Cyclase in Vasodilation to Sildenafil**

Incubation of retinal arterioles with the NOS inhibitor L-NAME for 30 minutes did not significantly alter basal tone (control: 67% ± 5% vs. L-NAME: 67% ± 5%, P = 0.08) but abolished the vasodilatory response to sildenafil (Fig. 1). In a separate series of studies, treating the vessels with the soluble guanylyl cyclase inhibitor ODQ did not alter the resting tone (control: 70% ± 2% vs. ODQ: 69% ± 2%, P = 0.14). However, ODQ abolished the vasodilatory response to sildenafil in a manner similar to L-NAME (Fig. 1).

**Role of ERK Signaling and Potassium Channels in the Vasodilatory Response to Sildenafil**

The ERK kinase inhibitor PD98059 abolished the vasodilatory response to sildenafil, except at the highest concentration (i.e.,...
Incubation with either the nonselective potassium channel blocker TEA or the selective K<sub>ATP</sub> channel blocker glibenclamide significantly inhibited the sildenafil-induced vasodilation (Fig. 2). PD98059, TEA, and glibenclamide did not change the basal tone (control: 67% ± 1% vs. PD98059: 65% ± 2%, P = 0.30; control: 70% ± 4% vs. TEA: 70% ± 4%, P = 0.61; control: 68% ± 4% vs. glibenclamide: 68% ± 4%, P = 0.90).

**Role of ERK Signaling and Potassium Channels in the Vasodilatory Response to NO**

The NO donor SNAP dilated the retinal arterioles in a concentration-dependent manner, and this dilation was not affected by PD98059. However, both TEA and ODQ inhibited retinal arteriolar dilation in response to SNAP in a similar manner (Fig. 3).

**Role of the PDE5 Pathway**

To determine the role of PDE5 in sildenafil-induced vasodilation, the following series of experiments were performed in the presence of l-NAME to eliminate the NOS-dependent pathway. As indicated in Figure 1, the vasodilation elicited by sildenafil was abolished by l-NAME. In the presence of l-NAME (30 minutes), the addition of SNAP (1 μM) produced a moderate dilation (26% ± 6%) of retinal arterioles within 1 minute. Subsequent administration of sildenafil produced concentration-dependent dilation of the vessels in a manner comparable to that observed in control conditions (i.e., without l-NAME; Fig. 4). This restored vasodilation was insensitive to PD98059 but was abolished by TEA (Fig. 4).

**DISCUSSION**

The reports of the clinical effects of sildenafil on ocular circulation are contradictory, and it is unclear whether this drug exerts a vasodilatory effect on retinal arterioles. In the present study, we used a videomicroscopic technique for diameter measurement and provide the first direct evidence that sildenafil produces dose-dependent dilation of retinal arterioles at concentrations between 1 ng/mL and 1 μg/mL. It has been
shown that the plasma sildenafil concentration can reach 0.5 to 0.7 μg/mL in human subjects within an hour after a single oral administration of 100 mg. Therefore, the concentrations of sildenafil used in the present study are clinically relevant. The threshold concentration for sildenafil to dilate retinal arterioles is at 10 ng/mL and the highest concentration (1 μg/mL) produces up to 30% maximal dilation. Although the magnitude of dilation might be modest, this vasomotor response is expected to have a significant impact on local retinal perfusion because blood flow, under constant driving pressure, is a function of the fourth power of the vessel radius. In contrast, it should be noted that sildenafil, at clinical doses, is not a potent vasodilator in retinal arterioles (Fig. 1). Therefore, its direct vasomotor actions are likely to be modulated and/or masked by various vasoregulatory feedback mechanisms in vivo, which may partly explain the inconsistent findings in hemodynamics and vascular responses to sildenafil in the human ocular circulation.

Based on the results derived from the present study, we propose that a signaling pathway exists for retinal arteriolar dilation in response to sildenafil, as shown in Figure 5 and discussed later herein. We first examined the possible role of the endothelium-derived vasodilator NO in sildenafil-induced vasodilation. We found that blockade of NO abolished the sildenafil-induced response, suggesting that NO released from NOS contributes mostly to the dilation of retinal arterioles. Our previous studies have shown that porcine retinal arterioles express endothelial NOS, supporting the possible role of endothelial NO signaling in response to sildenafil. Since activation of the soluble guanylyl cyclase/cGMP pathway is generally considered as a major vasodilatory mechanism for NO, it is expected that the selective soluble guanylyl cyclase inhibitor ODQ can inhibit sildenafil-induced dilation. Indeed, ODQ abolished the dilation of retinal arterioles to sildenafil in a manner comparable to that produced by L-NAME, indicating the pivotal role of soluble guanylyl cyclase/cGMP signaling in mediating NOS-dependent dilation in response to sildenafil in retinal arterioles (Fig. 5).

Of interest, ERK kinase inhibitor PD98059 significantly attenuated sildenafil-induced vasodilation, indicating the involvement of ERK signaling in this vasomotor response. Although ERK activation leading to increased NOS activity and subsequent rapid NO release has been reported, it also has been
shown that NO can induce ERK activation via a cGMP-dependent mechanism in various cell types, including the endothelium. Therefore, ERK signaling can be at the upstream or downstream of NOS. To determine the location of ERK in mediating sildenafil-induced vasodilation, we performed another series of experiments by examining whether the vasodilation elicited by NO (i.e., activation of cGMP pathway) is through ERK signaling. We found that the dilation of retinal arterioles to NO donor SNAP was insensitive to PD98059 (Fig. 3), suggesting that ERK signaling underlying sildenafil-induced vasodilation is at the upstream of NO (i.e., NOS activation) (Fig. 5).

Elevation of smooth muscle cGMP has been shown to activate potassium channels in some types of vasculatures, leading to vasorelaxation by the inhibition of transmembrane influx of calcium through voltage-gated calcium channels during membrane hyperpolarization. We have shown in other studies that potassium channels play an important role in the retinal arteriolar dilation in response to adenosine, lactate, and SNAP stimulation via the cGMP-mediated pathway. In the present study, the effect of potassium channel blockade on sildenafil-induced dilation (Fig. 2) was found to be comparable to that produced by l-NAME and ODQ (Fig. 1). It is possible that sildenafil induces a signaling pathway linking NO/cGMP to potassium channels. More specifically, we assumed that potassium channel activation is at the downstream of NO/cGMP signaling. If this were the case, we would expect to see the blockage of retinal arteriolar dilation to NO donors (via cGMP signaling) by both ODQ and TEA. Indeed, ODQ and TEA inhibited the dilation of retinal arterioles in response to SNAP in a similar fashion (Fig. 3). These results support the cardinal role of potassium channels in NO/cGMP-mediated, sildenafil-induced vasodilation (Fig. 5). It appears that the specific activation of $K_{ATP}$ channels is involved, since glibenclamide abolished the vasodilatory response to sildenafil (Fig. 2). These results are consistent with our previous findings showing that NO/cGMP-induced dilation in the porcine retinal arterioles is mediated by the opening of $K_{ATP}$ channels.

The complete inhibition of sildenafil-induced dilation by l-NAME is unanticipated, because sildenafil is a PDE5 inhibitor and is expected to act downstream of NO to elevate cGMP for vasodilation (Fig. 5), unless the source of cGMP is totally eliminated and the resting level of cGMP and/or the activity of PDE5 is too low to be functional. Since the resting level of cGMP is determined by the activity of both guanylyl cyclase (for synthesis) and PDE5 (for degradation), the slight effect of ODQ and l-NAME on resting tone and the failure of sildenafil to produce dilation in the presence of these antagonists indicates that the NO derived from NOS is the sole source for cGMP and that the resting level of NO/cGMP may have been too low to allow sildenafil to exert its vasodilatory influence via the inhibition of PDE5. Alternatively, the PDE5 may be inactive in retinal arterioles. Of interest, in the presence of l-NAME, the retinal arterioles dilated in response to sildenafil when exogenous NO (i.e., SNAP) was provided (Fig. 4). This result supports the functional activity of PDE5 in modulating NO-mediated vasodilation. It appears that a sufficient provision of NO, thus cGMP, to the vessel is essential in terms of allowing retinal arterioles to respond to sildenafil through the PDE5-inhibition pathway. Therefore, the release of NO from activated NOS plays not only a direct role in sildenafil-induced vasodilation, but also a permissive role in modulating this dilation via inhibition of PDE5. In contrast to NOS activation, this PDE5 inhibition pathway was insensitive to PD98059 (Fig. 4), suggesting that the vasodilation mediated by PDE5 inhibition is independent of ERK signaling. This finding is consistent with the idea that the vasodilation elicited by the NO/cGMP pathway (i.e., downstream from NOS) is not mediated by the ERK activation as shown in Figure 3. On the other hand, similar to the NOS pathway, the vasodilation through PDE5 inhibition was abolished by TEA (Fig. 4), indicating that potassium channels are the final effector for sildenafil-induced dilation by increased cellular cGMP through the pathways converging from NOS activation and PDE5 inhibition (Fig. 5).

One of the important findings and implications of the present study is that sildenafil did not evoke the classic PDE5-inhibition pathway for retinal arteriolar dilation unless a high level of cGMP was supplied from either the blood vessel per se (via endothelial NOS activation) or the surrounding parenchymal tissues. It has been shown that retinal neuronal cells express NOS and soluble guanylyl cyclase. Although the direct effect of NO on retinal arterioles is vasodilation, the increased parenchymal NO, and thus cGMP, has been shown to promote vasoconstriction by inhibiting the release of vasodilator epoxyeicosatrienoic acids from glial cells during neuronal activation (by flickering white light) in the intact retina. In this regard, the balance between vascular and parenchymal NO appears to determine the final vascular tone in intact retinal tissues. It is conceivable that under cardiovascular risks such as hyperlipidemia, diabetes, and hypertension (i.e., conditions in which endothelial NOS function is generally jeopardized), retinal arteriolar dilation to sildenafil may be directly compromised and the glial cell-mediated vasoconstriction may be promoted by increasing or prolonging the action of NO via PDE5 inhibition in parenchymal tissues. If similar conditions exist in the microcirculation of the optic nerve head, the net vasoconstriction may contribute to the development of the nonarteritic anterior ischemic optic neuropathy that has been described in association with sildenafil administration. In addition to the possible inhibition of PDE6, this may explain, in part, the transient visual disturbances associated with using sildenafil or other PDE5 inhibitors for the treatment of erectile dysfunction, especially in patients with cardiovascular risks.

In summary, the present study demonstrates that sildenafil, at clinical doses, elicits a modest dilation of porcine retinal arterioles via activation of NO through ERK signaling and the subsequent activation of guanylyl cyclase. The guanylyl cyclase/cGMP signaling triggers the opening of the $K_{ATP}$ channels for vasodilation. Moreover, the elevated cGMP, by endogenous or exogenous NO, plays a permissive role in sildenafil-induced dilation through the inhibition of PDE5 in an ERK-independent manner.

References