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The Role of cGMP in Ocular Growth and the Development of Form-Deprivation Myopia in Guinea Pigs

Fang Fang,1,3 Miaozen Pan,1,2 Tingting Yan,1,2 Yijin Tao,1,2 Hao Wu,1,2 Xing Liu,1,2 Jia Qu,1,2 and Xiangtian Zhou1,2

1School of Optometry and Ophthalmology and Eye Hospital, Wenzhou Medical University, Wenzhou, Zhejiang, China
2State Key Laboratory Cultivation Base and Key Laboratory of Vision Science, Ministry of Health of P. R. China, Wenzhou, Zhejiang, China
3Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, Hunan, China

Correspondence: Xiangtian Zhou, School of Optometry and Ophthalmology and Eye Hospital, Wenzhou Medical University, 270 Xueyuan Road, Wenzhou, Zhejiang 325003, China; zxt-dr@wz.zj.cn.
Jia Qu, School of Optometry and Ophthalmology and Eye Hospital, Wenzhou Medical University, 270 Xueyuan Road, Wenzhou, Zhejiang 325003, China; jqu@wz.zj.cn.
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PURPOSE. Development of myopia is associated with remodeling of the sclera, a tissue composed principally of collagen. Cyclic guanosine monophosphate (cGMP) regulates collagen synthesis in several organs; therefore, we investigated the effects of soluble guanylyl cyclase (sGC) stimulation and inhibition on refraction and ocular growth in guinea pigs under normal and form-deprived (FD) conditions.

METHODS. Retinal and scleral cGMP concentrations were measured in normal and monocularly FD guinea pigs at 2 days, 1 week, and 2 weeks of form deprivation and following 2 days recovery. Stimulation of sGC by BAY41-2272 and inhibition by NS-2028 were achieved by daily subconjunctival injection in normal and FD eyes. Refraction and axial parameters were measured at the commencement, middle, and cessation of the experiment. cGMP levels were also determined at the end of the experiment.

RESULTS. Retinal and scleral cGMP concentrations increased in FD eyes from 2 days to 2 weeks (P < 0.029). Levels decreased after 2 days of recovery (P < 0.003). Daily injections of BAY41-2272 induced a myopic shift (P < 0.001) and ocular elongation (P < 0.01) in normal animals, but did not alter myopia in FD eyes (P > 0.05). In contrast, daily injections of NS-2028 partially reduced myopic shifts (P < 0.012) and ocular elongation (P < 0.015) induced by form deprivation, but did not affect ocular growth and refraction in normal eyes (P > 0.05). Retinal and scleral cGMP levels were increased by BAY41-2272 in normal eyes and decreased by NS-2028 in FD eyes.

CONCLUSIONS. Changes in cGMP signaling contribute to myopic development. Thus, cGMP may be a potential therapeutic target for preventing/treating myopia.

Keywords: cGMP, BAY41-2272, NS-2028, form-deprived myopia, guinea pigs

Myopia, a mismatch between the refractive power of the optical system and the axial length of the eye, is one of the most common ocular diseases worldwide. The incidence of this visual disturbance is continually increasing, especially in East Asia where it reaches as high as 80% of the population.2,3 In the case of high myopia (≥−6.0 diopters [D]), pathological changes such as macular degeneration, subretinal hemorrhage, and retinal detachment can lead to permanent visual impairment and blindness.4,5 Therefore, myopia is a significant public health concern. However, the exact mechanism of myopic development and progression has not been fully clarified.

In most cases, excessive axial elongation of the vitreous chamber is the main structural change that occurs during development.5–7 This elongation causes the image to be focused in front of the retinal plane. It appears that active remodeling of the sclera, rather than passive stretching, facilitates the ocular enlargement.8–10 The sclera consists mainly of secreting fibroblasts dispersed in an extracellular matrix (ECM).9 The ECM serves as a fibrous, dense shell that constrains ocular growth. During myopic development, there is a decrease in scleral collagen and glycosaminoglycan content, a change in diameter and organization of the collagen fibrils, a loss of dry weight, and a reduction of scleral thickness.11–14

Despite our understanding of changes in the scleral ECM during myopic development, the initiators of these changes are relatively obscure. Recently, Wu et al. found that cyclic guanosine monophosphate (cGMP) levels increased in the posterior ocular tissue—including retina, choroid, and sclera—during myopic development induced by 1 to 3 weeks of form-deprivation in guinea pigs.15 Cyclic guanosine monophosphate (cGMP) is a ubiquitous cellular second messenger that is produced from guanosine triphosphate (GTP) by guanylyl cyclase (GC). It is an important cell-signaling molecule in many physiological events such as vasodilatation, neurotransmission, immunological regulation, wound healing, apoptosis, and gene regulation.16,17 Pharmacologically elevated cGMP levels inhibit collagen synthesis in normal dermal fibroblasts18 and keloid fibroblasts.19 Similarly, increasing cellular cGMP levels by a cGMP analog blocked TGF-β1-induced myofibroblast transformation, proliferation, and collagen synthesis in mouse cardiac fibroblasts.20 Together these findings suggest that the cGMP pathway may regulate collagen synthesis by scleral fibroblasts and thus control scleral remodeling and
cGMP Mediates Myopic Development in Guinea Pigs

**MATERIALS AND METHODS**

**Animals and FD Myopia (FDM)**

This study was approved by the Animal Care and Ethics Committee at Wenzhou Medical University (Wenzhou, China). The treatment and care of animals were conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research. Guinea pigs (n = 352; 3 weeks old) were raised in standard cages in a 12-hour light/dark cycle during the experiment. Food and water were available ad libitum. FDM was achieved by a latex facemask (Ou Jie, Suzhou, China) covering one eye and leaving the other eye, nose, and both ears freely exposed, as described previously.21,22 The facemasks were examined once daily to ensure that they were in place. After 2 weeks of form deprivation, myopic recovery was achieved by taking off the facemask for 2 days.

**Experimental Design**

In the first set of experiments, guinea pigs (n = 118) were randomly assigned to normal (NOR), FD, and recovery (REC) groups for analyzing changes in cGMP levels during form-deprivation and recovery. The right eyes of FD and REC groups were covered with the latex facemask. The FD group was divided into three subgroups that were FD for 2 days (n = 18), 1 week (n = 18), and 2 weeks (n = 17). The animals in the REC group (n = 17) were FD for 2 weeks, and then the facemasks were taken off for 2 days. The animals in NOR groups were used as age-matched controls and had no treatment for 2 days (n = 15), 1 week (n = 15), and 2 weeks (n = 18). cGMP concentrations in the retina and sclera were measured by radioimmunoassay (RIA) after the treatment.

In the second set of experiments, guinea pigs (n = 234) were allocated into NOR and FD groups for analyzing the effects of soluble guanylyl cyclase (sGC) stimulation and inhibition on both normal visual and myopic development. Each group was divided into noninjection, vehicle-injection, and drug-injection groups (sample size varied by group; see Table). Injections of the drugs or vehicle into the subconjunctival region were performed daily, and biometric parameters were measured at the commencement, middle, and cessation of the experiment. cGMP concentrations in the retina and sclera were also measured at the end of each drug treatment.

**Pharmacological Manipulation**

sGC activation was achieved by BAY41-2272 (Sigma-Aldrich, St. Louis, MO)23 and sGC inhibition was achieved by NS-2028 (Cayman, Ann Arbor, MI).24 The drugs were freshly prepared in dimethyl sulfoxide (DMSO) and then diluted 1000 times in 0.9% saline before each injection. The vehicle solution also contained 0.1% DMSO in 0.9% saline.

The drug solution (100 μL) was injected through the inferior palpebral subconjunctiva using a 26-gage disposable syringe once daily at 9 AM. The injections were performed in the right eyes of alert animals with help from an animal care assistant.

**Ocular Biometric Measurements**

Refraction (RE) was measured in the vertical pupil meridian by eccentric infrared photoretinoscopy.22,25 No general anesthesia was necessary for any of the measurement procedures because the animals were very docile. Room light was dimmed to approximately 5 lux to minimize the signal-to-noise ratio of the measurements. An average of 3 refraction readings for each eye was accepted for further analysis.

The corneal radius of curvature (CRC) of alert guinea pigs was measured with a keratometer (OM-4; Topcon, Tokyo, Japan) mounted with a +8 D lens.21,22 The average of three readings was recorded for further analysis.

After topical anesthesia with 0.5% proparacaine hydrochloride (Alcon, Purr, Belgium), ocular axial dimensions were measured by A-scan ultrasonography (AVISO Echograph Class I-Type Bat; Quantel Medical, Clermont-Ferrand, France) implemented with an 11-MHz transducer.21,22 Velocities of sound were assumed to be 1534 m/s for the aqueous and vitreous humor, and 1774 m/s for the lens, as described by Schaeffel and Howland.26 Recorded parameters included anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL). The ultrasound data were calculated from the average of eight separate measurements.

**cGMP Assay**

**Sample Preparation.** Samples were immediately obtained after euthanasia with pentobarbital sodium (130 mg/kg).15,27 Eyes were enucleated and placed on ice. The anterior part containing the lens was discarded (eye cup) and the retina was carefully separated under visual control of a dissecting microscope (Stemi 2000; Zeiss, Goettingen, Germany). Because of the loose adhesion between the retina and the RPE, the retina could be easily separated from the eye cup. The isolated retina was then washed gently to remove the few adherent RPE cells.28,29 Because the choroidal tissue adhered strongly to the sclera, cotton swabs were used to remove it from sclera under the dissecting microscope. The entire sclera and retina were weighed and homogenized with 2 mL ice cold acetate buffer (50 mM sodium acetate, 50 mM acetic acid, 4 mM ethylene-diaminetetraacetic acid, pH 4.75). After the addition of 2 mL dehydrated alcohol, the mixture was centrifuged at 1800g for 15 minutes. The supernatant was collected, and the precipitate

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**Table.** Drugs, Pharmacological Actions, Groups, and Sample Size of the Second Set of Experiments

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Effects on cGMP Levels</th>
<th>Groups</th>
<th>Noninjection</th>
<th>Vehicle Injection</th>
<th>Drug Injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAY41-2272</td>
<td>Increase</td>
<td>NOR</td>
<td>13</td>
<td>15</td>
<td>0.1 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FD</td>
<td>14</td>
<td>14</td>
<td>10 μM</td>
</tr>
<tr>
<td>NS-2028</td>
<td>Decrease</td>
<td>NOR</td>
<td>15</td>
<td>15</td>
<td>0.1 μM</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FD</td>
<td>16</td>
<td>15</td>
<td>10 μM</td>
</tr>
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</table>
was washed with 2 mL 75% alcohol and recentrifuged. The two supernatants were merged and dried at 60°C overnight. The residue was stored at 4°C until assayed.

**RIA for cGMP Levels.** The concentration of cGMP in the retinas and scleras was measured by RIA using a [125I] cGMP RIA kit (Izotop [Institute of Isotopes Ltd.], Budapest, Hungary). The residue was dissolved in 1 mL acetate buffer, and 0.1 mL of the solution was removed and mixed with acetylase reagent (triethylamine:acetic anhydride = 2:1), [125I]cGMP, and rabbit anti-cGMP serum, and incubated at 4°C overnight. After addition of the separating second antibody reagent containing donkey anti-rabbit serum, the mixture was incubated at room temperature for 10 minutes and then centrifuged at 1800 g for 15 minutes. The radioactivity was determined by counting for at least 60 seconds in a gamma scintillation counter (XH6080; Xi'An Nuclear Instrument Factory, Xi'An, China). Results were presented as fmol/mL.

**Statistical Analysis**

Statistical analyses were conducted using the statistical software (SPSS version 16.0; SPSS, Inc., Chicago, IL). Results were compared between treated and contralateral control eyes within the same group using paired sample t-tests. Independent sample t-tests were used for comparisons between control and vehicle groups as well as vehicle and drug groups. One-way ANOVA with Bonferroni correction was used for comparisons among the different drug concentration groups. All data in this study are shown as mean ± standard deviations unless otherwise stated. P values less than 0.05 were defined as significant.

**RESULTS**

**Retinal and Scleral cGMP Levels Increased in FD Eyes and Decreased in Recovery Eyes**

In the retina, cGMP levels in the NOR 2-week group increased significantly compared with the NOR 2-day and 1-week groups (**P < 0.01, one-way ANOVA, Fig. 1), indicating an age-related increase in the levels of cGMP. cGMP levels in deprived eyes were significantly higher than contralateral control eyes at 1 week of form deprivation (**P < 0.01, paired sample t-test). The cGMP level significantly increased in contralateral control eyes at FD 2-week group compared with the age-matched normal eyes (**P < 0.05, independent sample t-test). The cGMP level also increased significantly in contralateral control eyes at FD 2-week group compared with the age-matched normal eyes (**P < 0.05, independent sample t-test). The cGMP level significantly decreased and returned to the normal level by 2 days after unmasking (**P < 0.01, independent sample t-test). Right eye: form-deprived eye in FD groups. Left eye: control eye in FD groups.

**FIGURE 1.** Retinal cGMP levels. cGMP was measured in retinas of NOR and FD guinea pigs at 2 days, 1 week, and 2 weeks of FD and in animals at 2 days of REC after 2 weeks of FD. The cGMP level in the retinas of deprived eyes was significantly higher than in contralateral control eyes at 1 week of FD (**P < 0.01, paired sample t-test). The cGMP level significantly increased in deprived eyes after 2 days of FD compared with age-matched normal eyes (**P < 0.05, independent sample t-test). The cGMP level also increased significantly in contralateral control eyes at FD 2-week group compared with the age-matched normal eyes (**P < 0.05, independent sample t-test). Right eye: form-deprived eye in FD groups. Left eye: control eye in FD groups.

**FIGURE 2.** Scleral cGMP levels. cGMP was measured in scleras of normal and FD guinea pigs at 2 days, 1 week, and 2 weeks of FD and in animals at 2 days of REC after 2 weeks of FD. There was a significant difference between deprived eyes and contralateral control eyes in cGMP levels at 1 week of FD (**P < 0.05, paired sample t-test). The cGMP levels in both deprived and contralateral control eyes after 2 days of FD significantly increased compared with the age-matched normal groups (**P < 0.05, **P < 0.01, independent sample t-test). The cGMP level significantly decreased and returned to the normal level after 2 days of unmasking (**P < 0.01, independent sample t-test). Right eye: form-deprived eye in FD groups. Left eye: control eye in FD groups.
paired sample t-test, Fig. 1). cGMP levels in deprived eyes increased compared with age-matched normal eyes (FD 2-day versus NOR 2-day, $P = 0.024$; FD 1-week versus NOR 1-week, $P = 0.029$; FD 2-week versus NOR 2-week, $P = 0.002$; independent sample t-test, Fig. 1). The cGMP levels in the contralateral control eyes of FD 2-week animals increased compared with NOR 2-week animals ($P = 0.037$, independent sample t-test, Fig. 1). After 2 days recovery, the cGMP level in deprived eyes fell compared with FD 2-week eyes ($P = 0.003$, independent sample t-test, Fig. 1).

In the sclera, the cGMP levels were similar among normal groups of different ages ($P \geq 0.602$, one-way ANOVA with Bonferroni correction; Fig. 2). The cGMP levels in deprived eyes were higher than in contralateral control eyes at 1 week of

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**Figure 3.** Effects of BAY41-2272 injections on refraction, axial length, vitreous chamber depth, corneal radius of curvature, anterior chamber depth, and lens thickness in normal guinea pigs. Differences are between treated and untreated eyes. For normals, neither eye was treated and the differences are between right and left eyes. (A) Daily subconjunctival injections of BAY41-2272 (0.1 μM and 10 μM) induced relative myopia at 4 weeks ($P < 0.05$, **$P < 0.01$, independent sample t-test). (B) Daily subconjunctival injections of BAY41-2272 increased the axial length ($P < 0.05$, **$P < 0.01$), and (C) vitreous chamber depth (**$P < 0.01$). Daily subconjunctival injections of BAY41-2272 had no effect on (D) corneal radius of curvature, (E) anterior chamber depth, or (F) lens thickness in normal guinea pigs.
form deprivation ($P = 0.021$, paired sample $t$-test, Fig. 2), while they were similar between the eyes of the same animals in the FD 2-day and 2-week groups ($P \geq 0.324$, paired sample $t$-test, Fig. 2). cGMP levels in both deprived and contralateral control eyes were higher than age-matched normal eyes (NOR 2-day versus FD 2-day, $P \leq 0.01$; NOR 1-week versus FD 1-week, $P \leq 0.027$, NOR 2-week versus FD 2-week, $P < 0.001$; independent sample $t$-test; Fig. 2). After 2 days of recovery, cGMP levels in deprived eyes fell compared with the FD 2-week eyes (REC 2-day versus FD 2-week, $P < 0.001$, independent sample $t$-test, Fig. 2).

**BAY41-2272 Induced Myopia in Normal Eyes but Had No Effect on FD Eyes**

To control for a vehicle effect, comparisons were performed between vehicle control and drug-treatment groups to evaluate the effect of the drugs on biometric measurements. There were no differences in biometric measurements between the NOR and NOR+vehicle groups or between the FD and FD+ vehicle groups ($P \geq 0.452$, independent sample $t$-test, Figs. 3, 4, 6, 7). This similarity indicates that the vehicle had no effect on ocular biometric measurements.
In normal animals, 2 weeks of subconjunctival injections of 10 μM BAY41-2272 induced a greater refraction difference between treated and contralateral control eyes compared with DMSO–treated animals (P < 0.001, independent sample t-test, Fig. 3A and Supplementary Table S1). During this time, the interocular AL difference in the 10 μM BAY41-2272–treated group was more than in the DMSO–treated group (P = 0.005, independent sample t-test, Fig. 3B and Supplementary Table S1). After 4 weeks treatment, both 0.1 μM and 10 μM BAY41-2272 induced a significant myopic shift between treated and contralateral control eyes compared with DMSO–treated animals (0.1 μM BAY41-2272–versus DMSO–treated group, P = 0.001; 10 μM BAY41-2272 versus DMSO–treated group, P < 0.001; independent sample t-test, Fig. 3A and Supplementary Table S1). Simultaneously, the interocular AL difference in 0.1 μM BAY41-2272–treated group increased compared with the

**Figure 4.** Effects of BAY41-2272 injections on refraction, axial length, vitreous chamber depth, corneal radius of curvature, anterior chamber depth, and lens thickness in FD guinea pigs. Differences are between treated and untreated eyes. For FD group, neither eye was treated with BAY41-2272 and the differences are between form-deprived and contralateral eyes. Daily subconjunctival injections of 0.1 μM or 10 μM BAY41-2272 had no effect on the (A) refraction, (B) axial length, (C) vitreous chamber depth, (D) corneal radius of curvature, (E) anterior chamber depth, or (F) lens thickness in FD guinea pigs.
DMSO-treated group ($P = 0.01$; independent sample $t$-test, Fig. 3B and Supplementary Table S1). For the group treated with 10 μM BAY41-2272, the interocular differences in AL ($P < 0.001$, independent sample $t$-test, Fig. 3B and Supplementary Table S1) and VCD ($P < 0.001$, independent sample $t$-test, Fig. 3C and Supplementary Table S1) were greater than the DMSO-treated group. There were no changes in the CRC, ACD, or LT between the treated and contralateral control eyes or between drug and vehicle-treated groups (Figs. 3D–F and Supplementary Table S1).

We also investigated the effect of BAY41-2272 on FDM development. Daily injections with BAY41-2272 failed to alter the refraction, AL, or VCD changes induced by form-deprivation (Figs. 4A–C and Supplementary Table S2). Similarly, there were no changes in CRC, ACD, or LT between BAY41-2272-treated and contralateral control eyes or between BAY41-2272- and DMSO-treated groups (Figs. 4D–F and Supplementary Table S2). Taken together, BAY41-2272 treatment induced myopia and increased VCD and AL in normal eyes, but had no effect on myopic shift and associated axial elongation of FD eyes. Consistent with the changes in refraction and axial length, the retinal and scleral cGMP levels of treated eyes were greater than those in the contralateral control eyes in the NOR+ BAY41-2272 group (retina: $P = 0.014$; sclera: $P = 0.004$; paired sample $t$-test, Fig. 5). However, there was no difference in ocular cGMP levels between the eyes of the same animal in...
the FD+ BAY41-2272 group (retina: \( P = 0.491 \); sclera: \( P = 0.566 \); paired sample t-test, Fig. 5).

**NS-2028 Reduced Myopia in FD Eyes but Had No Effect on Normal Eyes**

In normal animals, daily NS-2028 injections for 2 and 4 weeks did not produce any change in refraction, AL, or VCD compared with the DMSO-treated group and to the contralateral control eyes (Figs. 6A–C and Supplementary Table S3). Similarly, NS-2028 injections did not affect CRC, ACD, or LT compared with the DMSO-treated group (Figs. 6D–F and Supplementary Table S3).

We also investigated the effect of NS-2028 on FDM development. Daily injections with only DMSO during the 2 weeks of form deprivation induced a myopic shift of \(-6.20 \pm 2.17\)D (difference between treated and contralateral control eyes). The interocular differences in refraction in both 0.1 \( \mu M \) and 10 \( \mu M \) NS-2028–treated groups were less than in the DMSO–treated group (0.1 \( \mu M \) NS-2028– versus DMSO–treated group, \( P < 0.001 \); 10 \( \mu M \) NS-2028– versus DMSO–treated group, \( P = 0.012 \); independent sample t-test, Fig. 7A and Supplementary Table S4). During this time, the interocular differences of AL in both 0.1 \( \mu M \) and 10 \( \mu M \) NS-2028–treated groups were less than in the DMSO–treated group (0.1 \( \mu M \) NS-2028– versus DMSO–treated group, \( P = 0.005 \); 10 \( \mu M \) NS-2028 versus DMSO–treated group, \( P = 0.01 \); independent sample t-test; Fig. 7B and Supplementary Table S4). Similarly, the interocular differences of VCD were less than in the DMSO–treated group (0.1 \( \mu M \) NS-2028– versus DMSO–treated group, \( P = 0.004 \); 10 \( \mu M \) NS-2028– versus DMSO–treated group, \( P = 0.015 \); one-way ANOVA with Bonferroni correction; Fig. 7C and Supplementary Table S4). There were no changes in CRC, ACD, or LT between NS-2028–treated and contralateral control eyes or between the NS-2028– and DMSO–treated groups (Figs. 7D–F and Supplementary Table S4). Taken together, NS-2028 had no effect on normal eyes, but reduced myopic refraction, VCD, and AL in FD eyes. Consistent with the changes in refraction and axial length, the retinal and scleral cGMP levels of treated eyes were less than those in the contralateral control eyes in the FD+NS-2028 group (retina: \( P = 0.001 \), sclera: \( P < 0.001 \); paired sample t-test, Fig. 8). Further, there were no significant differences in ocular cGMP levels between the eyes of the same animal in the NOR+NS-2028 group (retina: \( P = 0.662 \), sclera: \( P = 0.423 \); paired sample t-test, Fig. 8).

**DISCUSSION**

cGMP Signaling Plays an Important Role in the Development of Myopia

The principal finding in the present study is the association between changes in cGMP signaling and myopia development. Specifically, elevation of cGMP levels by the sGC stimulator BAY41-2272 induced axial elongation and myopic shift in normal eyes, but had no effect on FD eyes. Consistent with this finding, the decrease of the cGMP level by the sGC inhibitor NS-2028 partially reduced the myopic changes induced by FD, but had no effects on normal vision. These results suggest that there could be an upregulation of cGMP levels during myopic development, and this was confirmed in our cGMP measurements that showed a persistent increase in FD eyes. Following
Figure 6. Effects of NS-2028 on refraction, axial length, vitreous chamber depth, corneal radius of curvature, anterior chamber depth, and lens thickness in normal guinea pigs. Differences are between treated and untreated eyes. For normals, neither eye was treated, and the differences are between right and left eyes. Daily subconjunctival injections of NS-2028 had no effect on (A) refraction, (B) axial length, (C) vitreous chamber depth, (D) corneal radius of curvature, (E) anterior chamber depth, or (F) lens thickness in normal guinea pigs.
FIGURE 6. Continued.

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D

Difference in CEC (mm)

NOR  DMSO  NS-2028 0.1 μM  NS-2028 10 μM

E

Difference in ACD (mm)

NOR  DMSO  NS-2028 0.1 μM  NS-2028 10 μM

F

Difference in LT (mm)

NOR  DMSO  NS-2028 0.1 μM  NS-2028 10 μM
facemask removal, there was a rapid reduction in cGMP levels. These data are consistent with findings by Wu et al. Taking together, the drug-induced changes in cGMP levels and refraction suggest that changes in myopia may be related to differences in basal cGMP levels. In normal animals, cGMP levels were low, and they increased during myopic development. Therefore, BAY41-2272 treatment only increased cGMP levels if the baseline levels were low. This was associated with myopia induction, but it had minimal effects in FD animals in which the baseline cGMP levels were elevated. In contrast, NS-2028 treatment in FD animals decreased the elevated cGMP levels to attenuate the myopia, but it failed to further decrease the already low cGMP levels in untreated eyes. Such dependence of these two opposing drug effects on baseline cGMP levels is similar to that described for pharmacological “ceiling” and “floor effects.”

Furthermore, the pharmacologically induced myopia in normal animals suggests that cGMP signaling contributes to normal emmetropization. Increases in cGMP and form deprivation can both induce axial myopia even though the increases associated with form deprivation are different from those caused by a cGMP activator. This suggests that elevated cGMP levels play a causal role during the development of myopia rather than being the outcome of myopia. Interestingly, the pharmacological reduction of FDM by sGC inhibition and the absence of any associated effect on normal eye growth suggest a promising therapeutic modality. Thus it may be possible to target cGMP levels for the prevention and/or treatment of myopia without affecting normal visual development. The onset of myopia usually occurs during the juvenile years when both normal emmetropization and myopigenesis occur. The apparent absence of effects by sGC inhibition on normal eye growth suggests that it may be a safe way to prevent and/or treat myopia.

Involvement of cGMP signaling in myopic development is also consistent with previously reported findings for other pharmacological agents. Intravitreous injections of the nitric oxide synthase (NOS) inhibitor L-NAME inhibited FDM and lens-induced myopia in chicks but had no effect in normal chicks. A primary target of the NO produced by NOS is sGC. Thus, inhibition of experimental myopia by L-NAME may be mediated by the sGC/cGMP signaling pathway. Furthermore, nonselective and M1-selective muscarinic antagonists (e.g., atropine and pirenzepine) are effective in slowing the axial elongation and the myopic shift in humans and in animal models. This indicates that one or more of the five muscarinic receptor subtypes are involved in ocular growth and myopia. Atropine can antagonize the pharmacological enhancement of cGMP levels. Therefore, it is possible that muscarinic antagonists inhibit myopic development via cGMP signaling in keeping with our results.

A Hypothesis for the Role of cGMP in Refractive Development

The exact mechanism by which cGMP contributes to the onset of FDM has not yet been elucidated. The involvement of the cGMP signaling in myopic development is most likely localized in the sclera where relatively high concentrations of BAY41-2272 and NS-2028 were delivered by subconjunctival injection. Accordingly, elevation of cGMP levels through activation of sGC by BAY41-2272 resulted in the onset of myopia in normal eyes. Consistent with these data, the reduction of cGMP levels by NS-2028 in FD eyes resulted in a reduction of myopia. In humans and other mammals, scleral thinning is associated with a remarkable loss of collagen, ultimately resulting in alterations in ocular growth and myopic development. Pharmacological increases in cGMP levels are capable of inhibiting collagen synthesis in different fibroblasts from humans and animals, including heart, skin, and kidney. Based on these findings, the cGMP signaling control of ocular growth and refractive development may be mediated by the regulation of collagen synthesis of scleral fibroblasts. The molecular mechanisms of the regulatory effect of cGMP signaling on myopic development have not been studied. cGMP-dependent protein kinase (PKG) is activated by cGMP, and a previous study showed that the cGMP/PKG signaling inhibited TGF-β1-induced myofibroblast transformation, proliferation, and collagen synthesis in mouse cardiac fibroblasts. Thus, sGC/cGMP/ PKG signaling may be of importance in controlling collagen synthesis during myopic development.

We have confirmed a role for cAMP signaling in myopic development. Similar to cGMP, increases in cAMP levels also induced myopia in normal guinea pigs, while a decline in cAMP levels prevented FDM. The involvement of cGMP signaling may be complex due to the possibility of interaction with the cAMP pathway control through crosstalk, as reported within ocular and other tissues. This type of interaction represents an alternative mechanism by which cGMP signaling could contribute to myopic development.

cGMP is extensively distributed in the ocular tissues, including iris, aqueous humor, choroids, and retina; therefore, there may be other cGMP-regulated mechanisms that contribute to the myopic changes. For example, cGMP lowers intraocular pressure (IOP) by decreasing aqueous humor formation. There are conflicting negative and supportive reports on the relationship between increases in IOP and myopia. Thus, the cGMP pathway may, in part, control ocular growth and myopic development by regulating the IOP. Furthermore, cGMP in the retina participates in vision transduction. The cGMP level in the retina decreases due to the light-dependent bleaching of rhodopsin followed by the activation of a cGMP phosphodiesterase (PDE). In the dark, retinal cGMP levels are high due to the constitutive activity of particulate GC. Consistent with this, increases in retinal cGMP levels during form-deprivation and declines during recovery most likely result from changes in light penetration to the retina. Retinal sGC is mainly located in cones, bipolar, and amacrine cells. Thus, the changes in retinal cGMP levels after BAY41-2272 and NS-2028 treatment probably result mostly from the alteration of sGC activity in these cells. Because the inner retina appears to be important for normal emmetropization, it is also plausible that the sGC/cGMP signaling in the retina is involved in myopic development. Taken together, cGMP may participate in myopic development through multiple alternative routes rather than through a single definitive mechanism. Obtaining additional insight into this question remains a topic for future study.

Yoking Effect During FD

Wu et al. reported that cGMP levels are upregulated in FD eyes as a whole, but they did not present any data on contralateral control eyes. We found that the ocular cGMP level increased not only in deprived eyes, but also in contralateral control eyes compared with normal eyes (Figs. 1, 2). However, only deprived eyes developed myopia. There are two questions of interest regarding the changes in cGMP levels in the contralateral control eyes. The first one is how the yoking effect occurs. Other studies have also demonstrated a yoking effect between treated and untreated fellow eyes involving monocular lens removal in mice; monoclonal injection of endotoxin in rats; monoclonal FD in chicks; and monocular lens induction in tree shrew. These findings further indicate that the yoking effect is prevalent in many conditions. Taken together, the yoking effect indicates that the
FIGURE 7. Effects of NS-2028 on refraction, axial length, vitreous chamber depth, corneal radius of curvature, anterior chamber depth, and lens thickness in FD guinea pigs. Differences are between treated and untreated eyes. For FD group, neither eye was treated with NS-2028, and the differences are between form-deprived and contralateral eyes. (A) Daily subconjunctival injections of NS-2028 reduced myopic shift induced by form-deprivation (*P < 0.05, **P < 0.01, independent sample t-test), (B) Daily subconjunctival injections of NS-2028 also decreased the axial length.
(*P < 0.05, **P < 0.01) and (C) vitreous chamber depth (*P < 0.05, **P < 0.01) in FD guinea pigs. Daily subconjunctival injections of NS-2028 had no effect on (D) corneal radius of curvature, (E) anterior chamber depth, or (F) lens thickness in FD guinea pigs.

Figure 7. Continued.
eyes are not completely functionally independent from one another. While the mechanism is not yet understood, it may be associated with neuronal or humoral factors, movement synergy, and/or ocular accommodation.

The second question is why changes in cGMP level in the contralateral control eye that are similar to those in the experimental eye do not cause myopia development. This difference suggests that not all changes in cGMP level are involved in myopia. Thus, different mechanisms may be engaged under normal and pathological situations such as monocular FD. As shown in our study, the same drug has different modes of action in normal vision and myopic development. Specifically, the increase in cGMP level by BAY41-2272 induced myopia in normal eyes, but did not affect the FDM; whereas the reduction in cGMP level by NS-2028 retarded the FDM, but did not affect the normal control eyes. Similarly, atropine also induces different effects on normal axial growth and FDM.

Another possibility is that the monocular facemask alters more than one variable in the treated eye. The covered eye is not only form-deprived, but it also receives decreased illumination, which may result in different changes. We presume that some of these changes can induce myopia, but others may have no relationship with the development of myopia. Furthermore, there is a transient increase in cGMP level in the deprived eye compared with the contralateral control eye within 1 week of FD, during which over half of the final degree of myopia was induced. This demonstrates that transient cGMP differences between two eyes may be one of the driving forces that promotes the myopic progression and thus is of greater importance than the absolute level during the myopic development.

**CONCLUSIONS**

In summary, we found that retinal and scleral cGMP levels were increased in FD eyes compared with normal control eyes. More importantly, the upregulation of the cGMP level by sGC activation with BAY41-2272 accelerated ocular growth and induced myopia in normal guinea pigs. Further, inhibition of sGC activity by NS-2028 reduced cGMP levels and attenuated the myopic changes in FD animals. These findings indicate that the cGMP pathway plays a critical role in the control of myopic development. Further research on the role of changes in cGMP signaling during myopia development may reveal potential drug targets for myopia prevention and/or treatment.

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**Figure 8.** Retinal and scleral cGMP levels in both eyes of NOR and FD animals at the end of NS-2028 10 μM treatment. Daily subconjunctival injections of NS-2028 10 μM reduced retinal (A) and scleral (B) cGMP levels in FD animals (**P < 0.01, ***P < 0.001, paired sample t-test), but had few effects on those in normal animals (NOR+NS-2028 10 μM, n = 9; FD+NS-2028 10 μM, n = 10).
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