Effects of Dopaminergic Agents on Progression of Naturally Occurring Myopia in Albino Guinea Pigs (Cavia porcellus)

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PURPOSE. Disruption of dopaminergic signaling has been implicated in the abnormalities of ocular development in albinism, and many experiments have shown that retinal dopamine is a major regulator of postnatal eye growth and myopia in animal models. Therefore, in the present study we investigated whether progressive myopia, which can occur in albino guinea pigs without experimental manipulation of visual conditions, is affected by dopaminergic agents.

METHODS. Two-week-old albino guinea pigs, selected for being myopic (range refractive error [RE], −2 to −10 dioptries [D]), received unilateral peribulbar injections of apomorphine (nonselective dopamine receptor agonist; 0, 7.5, 25, 75, 250, 750, and 2500 ng; n = 112), SKF38393 (D1-like agonist; 0, 10, 100, 1000 ng; n = 63), SCH23390 (D1-like antagonist; 0, 2500 ng; n = 27), quinpirole (D2-like agonist; 0, 10, 100, 1000 ng; n = 58), or sulpiride (D2-like antagonist; 0, 2500 ng; n = 24) once a day for four weeks. One noninjected group (n = 19) served as untreated control. Refractive states and axial dimensions of the eyes were measured without cycloplegia or general anesthetic, using eccentric infrared photoretinoscopy and A-scan ultrasonography, respectively, before treatment, and after 2 and 4 weeks of treatment. The main drug effects were analyzed by paired t-test or 2-way repeated measures ANOVA, as required.

RESULTS. The naturally occurring progression of myopic RE was inhibited by apomorphine at relatively high doses (250 and 750 ng), SKF38393 at 100 ng (D1-like agonist), and sulpiride at 2500 ng (D2-like antagonist), but promoted by apomorphine at a lower dose (25 ng), quinpirole at 100 ng (D2-like agonist), and SCH23390 at 2500 ng (D1-like antagonist). All drugs affected primarily vitreous chamber depth, rather than anterior segment dimensions.

CONCLUSIONS. Our data suggest that the activation of D1-like receptors inhibits, whereas activation of D2-like receptors promotes, progressive myopia in this animal model. The robust effects of antagonists suggest that ocular dopamine receptors in these albinos may be in a chronic state of partial excitation. The precise location and identity of the receptors responsible for these effects remain to be determined.

Keywords: refractive error, axial length, apomorphine, dopamine receptor, SKF38393, SCH23390, quinpirole, sulpiride

Myopia (near- or short-sightedness) is a common and clinically significant ocular disorder; its cause remains unclear, but laboratory studies in experimental animal models have provided many insights. Increased outdoor time is associated with a reduction in the prevalence of myopia, an effect that could be due to exposure to the relatively high intensity of outdoor light.1 High illumination levels retard experimental myopia in chicks2,3 and in some individual rhesus monkeys.4,5

Because retinal dopamine synthesis and release increase as a function of light intensity, in mammals, such as rats6 as well as in chicks,7 the retinal dopaminergic system has been studied extensively for its potential role in myopia inhibition.8,9 In form-deprivation myopia (FDM), growth of the form-deprived (occluded) eye can be inhibited by dopamine itself in rabbits,10 or by the dopamine-like agonist, apomorphine, in chicks.11–13 In negative-lens–induced myopia (FDM), retinal dopamine levels drop14 just as they do in FDM.15 However, the changes in dopamine metabolism in LIM are not as robust as those in FDM.16 and LIM has been reported to be inhibited by apomorphine in chicks.17,18 but not in guinea pigs.15 A dopamine precursor, levodopa (L-DOPA), inhibits FDM in pigmented guinea pigs,19 whereas a broad-spectrum dopamine agonist, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene

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refraction in this interesting mammalian model. The complexity of dopaminergic control of eye growth and role for dopaminergic signaling, while revealing the potential dopamine signaling, and, therefore, can be exacerbated or caused by an albinism-related deficiency in retinal or ocular progressive myopia, which occurs in albino guinea pigs, endogenous agonist (dopamine) itself elicits such effects.

Like and D2-like antagonists to determine whether the these two receptor families, and second, we used selective D1-(apomorphine) could have caused opposite effects through affinity D2-like receptors at lower doses. This hypothesis was further. These results suggested that the opposite effects of apomorphine were due to activation of the relatively low-affinity D1-like receptors at higher doses, and of the higher-affinity D2-like receptors at lower doses. This hypothesis was tested in two ways: first, we used selective D1-like and D2-like receptor agonists to test whether the exogenous agonist (apomorphine) could have caused opposite effects through these two receptor families, and second, we used selective D1-like and D2-like antagonists to determine whether the endogenous agonist (dopamine) itself elicits such effects.

In the current study, therefore, we investigated whether progressive myopia, which occurs in albino guinea pigs, without experimental manipulation of viewing conditions, is caused by an albinism-related deficiency in retinal or ocular dopamine signaling, and, therefore, can be exacerbated or ameliorated by treatment with DAs. Our results confirmed a role for dopaminergic signaling, while revealing the potential complexity of dopaminergic control of eye growth and refraction in this interesting mammalian model.

**Materials and Methods**

**Animals**

We obtained 2-week-old albino guinea pigs (*Cavia porcellus* the English short-hair stock, *n* = 305) from the laboratory animal center at Zhejiang University in Hangzhou, China. The large number of animals and sizes of our experimental groups (*n* = 11–18) reflect the fact that eye growth was more variable in these albino animals (see Results) than in wild-type populations. We used the minimum number of animals required to obtain statistically significant results, and stopped using more animals in any experimental group as soon as the results reached significance. Our previous studies showed that the average RE of these animals’ eyes was −6.0 ± 1.50 D at 2 weeks of age. Animals with myopic RE (from −2.00 to −10.00 D) were selected, because their myopic REs were within the 90% confidence interval around the mean; the mean RE was approximately −6 D in each experimental group at the start of the experiment, and the pretreatment differences in RE between groups, and between treated and fellow eyes in each group, were statistically insignificant (data not shown). The animals were reared under a 12-hour light/12-hour dark cycle, lights on at 08:00, in the laboratory animal center facilities. Illuminance at the cage floor was approximately 300 lux, which is reported to be sufficient for animal care and still not to cause retinal degeneration in albino animals. Visual exposure was limited to the cage environment, as described previously. Room temperature was kept at 25°C. The animals had free access to standard food and water, and fresh vegetables were provided twice a day. The use of animals for these studies was approved by the Animal Care and Ethics Committee at Wenzhou Medical College, Wenzhou, China. All procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**DAs and Peribulbar Injections**

All drugs used in this study (nonselective dopamine receptor agonist apomorphine, D1-like receptor agonist SKF38393, D1-like receptor antagonist SCH23390, D2-like receptor agonist quinpirole, and D2-like receptor antagonist sulpiride) were obtained from Tocris (Glasgow, UK). Using a 0.45 × 16-mm syringe cannula with a 25-gauge needle, 100 μL of vehicle containing various amounts of the tested drug (Table 1) was injected gently into the peribulbar space (more or less equivalent to “subconjunctival space,” but deeper in the orbit) through the lower conjunctival sac, under topical anesthesia with one drop of 0.5% proparacaine hydrochloride (Alcon, Puurs, Belgium). The vehicle for all drugs except sulpiride was Milli-Q water with 1 mg/mL ascorbic acid (Sigma-Aldrich Corporation, St. Louis, MO, USA) as anti-oxidant. Sulpiride was predissolved in dimethylsulfoxide (DMSO; Sigma-Aldrich Corporation), and then diluted in Milli-Q water to a final concentration of 0.1% DMSO. All injections were administered to the right eye of each animal, under dim red light (to avoid decomposition of the drugs by light during the procedure), once daily (9:00 AM) for four weeks. The left eyes remained untreated.

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**Table 1. Drugs Used in This Study and Their Observed Pharmacological Actions on Myopia Development**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Type</th>
<th>Dose, ng/Injection</th>
<th>Sample Size, <em>n</em></th>
<th>Effect on Myopia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apomorphine</td>
<td>Nonselective agonist</td>
<td>0, 7.5, 25, 75, 250, 750, 2500</td>
<td>18, 15, 17, 16, 15, 15</td>
<td>Promote and inhibit</td>
</tr>
<tr>
<td>SKF38393</td>
<td>D1 agonist</td>
<td>0, 10, 100, 1000</td>
<td>16, 15, 16, 16</td>
<td>Inhibit</td>
</tr>
<tr>
<td>SCH23390</td>
<td>D1 antagonist</td>
<td>0, 2500</td>
<td>12, 15</td>
<td>Promote</td>
</tr>
<tr>
<td>Quinpirole</td>
<td>D2 agonist</td>
<td>0, 10, 100, 1000</td>
<td>12, 16, 15, 15</td>
<td>Promote</td>
</tr>
<tr>
<td>Sulpiride</td>
<td>D2 antagonist</td>
<td>0, 2500</td>
<td>11, 13</td>
<td>Inhibit</td>
</tr>
</tbody>
</table>

Dose refers to the amount of the drug administered to the guinea pigs by peribulbar injection per day; the corresponding numbers of animals tested at each dose (sample sizes) are listed in the same sequence. The effects of the drugs are those observed in this study.
Experimental Protocols

Given our hypothesis that progressive myopia in albino guinea pigs is due to a deficiency in dopamine synthesis and release in the eye (probably in the retina), this study had three main aims.

Aim 1 was to determine whether progressive myopia in albino guinea pigs is prevented by releasing the hypothetical deficiency of dopamine release in the retina with an exogenous dopamine-receptor agonist. For this, we used the nonselective dopamine-receptor agonist apomorphine. We tested a wide range of doses (Table 1), because our preliminary data showed a biphasic effect on myopia progression ( unlike the monophasic effect that we observed previously in pigmented guinea pigs15), the effect of apomorphine observed in some other studies was biphasic, and the affinity of apomorphine differs in different dopamine receptor families.26–28 The treated eyes of the vehicle group (n = 16) received vehicle alone. To determine whether some part of the intrinsic growth-regulating signaling cascade involved activation of D1-like receptors, we applied the D1-like receptor antagonist, SCH23390, at the dose of 2500 ng (n = 15), and the eyes of animals in the untreated control group were not injected (n = 19).

Aim 2 was to learn whether any effect of apomorphine (in Aim 1) was due to activation of dopamine D1-like receptors. For this we administered three different doses of the D1-like receptor agonist, SKF38395: 10 (n = 16), 100 (n = 15), and 1000 (n = 16) ng in the ascorbic acid vehicle, while “treated” eyes in the vehicle group (n = 16) received vehicle alone. To determine whether some part of the intrinsic growth-regulating signaling cascade involved activation of D1-like receptors, for example by sustained release of endogenous dopamine, we applied the D1-like receptor antagonist, SCH23390, at the dose of 2500 ng (n = 15) in vehicle; again the vehicle group (n = 12) received vehicle alone. Details of drug doses, sizes of treatment groups, and effects on myopia progression are found in Table 1.

Aim 3 was to learn whether the dopamine D2-like receptor family was involved in the control of axial eye growth. Using a strategy analogous to that used in Aim 2, we administered three different doses of the D2-like receptor agonist, quinpirole: 10 (n = 16), 100 (n = 15), and 1000 (n = 15) ng in ascorbic acid vehicle; and the “treated” eyes of the vehicle group (n = 12) received vehicle alone. Similarly, to determine whether some part of the intrinsic growth-regulating signaling cascade involved activation of D2-like receptors by sustained release of endogenous dopamine, we injected the D2-like receptor antagonist sulpiride at a dose of 2500 ng in 0.1% DMSO (n = 13), while the treated eyes of the vehicle group received 0.1% DMSO alone (n = 11). For details see Table 1.

Biometry

Refractive errors were measured in the vertical pupil meridian using an eccentric infrared photorefractor, as described previously, at a camera distance of one meter.29 Alert guinea pigs are inherently cooperative, and it was easy to align their heads manually without any medication until the pupils were clearly visible in the video frame. It was not necessary to refract under cycloplegia, because a trial experiment using ten 3-week-old guinea pigs showed no significant difference between the values obtained for the same eyes, before or after cycloplegia; also, the statistics of repeated measurements show that the precision of measurement by the autorefractor is ±0.25 D (SD; data not shown). The room light was dimmed to approximately 5 lux ambient illuminance. Three readings of the RE in the vertical meridian were recorded for each eye, and the set of mean values was used for statistical analyses. We measured corneal curvature (and, thus, tested for astigmatism) in alert animals, using a modified keratometer (OM-4; Topcon, Tokyo, Japan) provided with a +8.0D lens, as described previously.30 The corneal radii of curvature along the two perpendicular meridians of each eye were measured three times and then averaged. The axial dimensions of the eyes were measured in alert, unanesthetized animals using A-scan ultrasonography (11 MHz, AVISO Echograph Class IType Bat; Quantel Medical, Clermont-Ferrand, France) on the same day as the REs were measured. The cornea was anesthetized topically with one drop of 0.5% proparacaine hydrochloride (Alcon). The velocities of sound were taken as 1534 m/sec in the aqueous and vitreous humors, and 1774 m/sec in the lens.23 To adapt the probe to the size of the guinea pig eye, a stand-off rubber tube was attached to the probe’s tip, as described by Schaeffel and Howland31 for use in chicks. The recorded parameters included anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and axial length (AL; distance from anterior surface of cornea to vitreal surface of retina). Each eye was measured at least eight times, and the average of those values was taken as a single value for further analyses.

All measurements were performed three times, at 2-week intervals, before the treatment started (“0 weeks”), and again after 2 and 4 weeks of treatment.

Statistical Analyses

All data are reported as mean ± SD, except that the error bars in Figures denote SEM. At the beginning of each experiment, the biometric data for right and left eyes of individual animals were compared using paired t-tests. To identify the treatment effects by comparing the different groups, interocular differences (i.e., the differences between the treated and fellow eyes) were calculated for all measured ocular parameters. Comparisons between vehicle groups and control groups were performed using 2-way repeated-measures ANOVA. To determine whether treatment of one eye affected dimensions in the fellow eye, the results were compared among groups injected with the same drug, but at different doses and in different vehicles, after four weeks of treatments, using 1-way ANOVA with Bonferroni post hoc correction. Paired t-tests were used to analyze differences between treated and control eyes of each animal in the same experimental group, after 4 weeks of treatment, and t-tests for two independent samples were used to analyze differences between two experimental groups when only one dose of a given agent was administered. Normality of distribution was confirmed for all data sets that were compared by these parametric tests. A P value of less than 0.05 was considered significant.

Results

At the beginning of each experiment, there were no differences in RE, corneal curvature (CC), or AL between the right and left eyes of individual animals (P > 0.05, paired t-tests), nor between the different groups of drug-treated eyes. In the control groups, the variance (SD) of refractions often increased with age during the 4 weeks of treatment, perhaps because the process of emmetropization was not tightly controlled in these animals (Table 2).

Effects of Vehicle on the Drug-Treated and Contralateral Eyes

Before undertaking the definitive data-analysis, we used 2-way ANOVA to test for drug effects on fellow eyes. In the case of all drugs, doses administered, and parameters measured, we found no significant effects on the fellow eyes; that is, ANOVA revealed no significant differences in any parameter measured, among fellow eyes in different groups, regardless of the drug
Dopaminergic Agents Effects on Myopia

Effects of Two Vehicles (Ascorbic Acid, DMSO) on RE and Vitreous Chamber Depth, Compared to Untreated Controls

<table>
<thead>
<tr>
<th>Time Points of Treatment</th>
<th>Normal Control, n = 19</th>
<th>Ascorbic Acid, n = 58</th>
<th>DMSO, n = 11</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refractive error, D</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>−5.97 ± 1.63</td>
<td>−5.80 ± 1.49</td>
<td>−5.79 ± 1.32</td>
</tr>
<tr>
<td>2 wk</td>
<td>−6.88 ± 2.16</td>
<td>−6.86 ± 2.24</td>
<td>−6.73 ± 1.93</td>
</tr>
<tr>
<td>4 wk</td>
<td>−7.67 ± 2.84</td>
<td>−7.54 ± 3.08</td>
<td>−7.48 ± 2.09</td>
</tr>
<tr>
<td>2-way ANOVA</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Vitreous chamber depth, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 wk</td>
<td>3.38 ± 0.15</td>
<td>3.37 ± 0.14</td>
<td>3.37 ± 0.14</td>
</tr>
<tr>
<td>2 wk</td>
<td>3.42 ± 0.17</td>
<td>3.41 ± 0.16</td>
<td>3.41 ± 0.16</td>
</tr>
<tr>
<td>4 wk</td>
<td>3.46 ± 0.20</td>
<td>3.46 ± 0.19</td>
<td>3.47 ± 0.19</td>
</tr>
<tr>
<td>2-way ANOVA</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

The data are expressed as the means ± SD. In each group, statistical analysis using 2-way ANOVA showed that the different vehicles did not have significantly different effects on RE in the “treated” (right) and fellow “control” (left) eyes, n.s., not significant. 0 wk, at the start of the experiment, before drug treatment (age, 2 weeks); 2 wk, 2 weeks after starting drug treatment (age, 4 weeks); 4 wk, 4 weeks after starting drug treatment (age, 6 weeks).

Effects of Apomorphine on Refractive Development in Myopic Albino Guinea Pigs

Growth and refraction in eyes treated with apomorphine for 4 weeks responded biphasically with respect to dose, as shown by differences in RE and VCD between the treated and fellow eyes (1-way ANOVA, P < 0.001; followed with Bonferroni’s correction; Fig. 1). The peaks of the biphasic response curves were at 25 and 250 ng of apomorphine.

At the lower doses, the development of myopia appeared to be promoted slightly by apomorphine. While the effects revealed by ANOVA were not statistically significant, a 2-tailed paired t-test revealed that the treated eyes were consistently larger and more myopic than the untreated fellow eyes (line graphs, Fig. 1A).

At the higher doses, in contrast, the myopic shift in RE was inhibited (P = 0.014), as were the corresponding increases in vitreous chamber (P = 0.013). The interocular differences of opposite sign in RE and VCD at the two optimal doses were statistically significant (P < 0.001, each).

Apomorphine treatment also significantly affected CC (P = 0.01), ACD (P = 0.009), and LT (P = 0.003), in different ways at higher versus lower doses (data not shown). Specifically, 250 ng apomorphine inhibited, whereas 25 ng promoted, the developmental myopic shifts in these parameters (CC, P = 0.024; ACD, P = 0.024; LT, P = 0.009). Compared to the lower dose, the higher dose caused less astigmatism (i.e., less difference of corneal curvature along the horizontal [H] versus vertical [V] meridian; [H−V] = 0.11 ± 0.10 mm at 250 ng vs. 0.14 ± 0.11 mm at 25 ng). The higher dose also caused greater ACD, but lesser LT (0.10 ± 0.16 mm at 250 ng vs. 0.04 ± 0.055 mm at 25 ng), suggesting that apomorphine increased ACD at the expense of LT.

Effects of Selective Dopamine D1-Like Receptor Ligands on the Development of Myopia

The selective D1-like receptor agonist, SKF38395, caused statistically significant differences between vehicle-treated and fellow eyes at most doses (Fig. 2). Four weeks of treatment at intermediate doses (10 and 100 ng) significantly inhibited the myopic shift in RE (F[3,62] = 5.623, P < 0.001; Fig. 2A); 100 ng SKF38395 also reduced axial elongation (VCD, P = 0.002; Fig. 2B) and significantly altered the interocular differences in RE and VCD compared to those in the vehicle subgroup (RE, P = 0.002; VCD, P = 0.007; Figs. 2A, 2B). At a lower dose of this agonist, 10 ng, the interocular difference in RE was small but statistically significant (P = 0.01), whereas the difference in VCD was not (P = 0.095). In contrast, SKF38393 had no effects on RE, VCD, or AL at 1000 ng, and no effects on anterior segment dimensions at any dose (data not shown).

In contrast, the selective D1-like receptor antagonist, SCH23390, promoted excessive eye growth and myopia development. Four weeks of treatment with this agent caused a myopic shift in refraction (RE, F[1,28] = 17.325, P = 0.001, Fig. 3A) and enhanced axial eye elongation (VCD, F[1,28] = 4.572, P = 0.041, Fig. 3B), compared to vehicle alone. After 2 weeks of treatment, the interocular difference in RE was −1.09 ± 0.78 D (P = 0.013), but the interocular differences in VCD and AL were not significantly affected (VCD, 0.04 ± 0.04 mm, P = 0.111; AL, 0.04 ± 0.04 mm, P = 0.089). After 4 weeks of treatment, the interocular difference in RE reached −1.64 ± 0.56 D (P < 0.001), and the interocular differences in VCD and AL, both = 0.07 ± 0.05 mm, reached significance (P = 0.002 each).

Effects of Selective Dopamine D2-Like Receptor Ligands on the Development of Myopia

Quinpirole, a dopamine D2-like receptor agonist, caused statistically significant, dose-related enhancements of myopia...
RE and VCD (Figs. 4A, 4B; paired t-test). Furthermore, the effects of 100 ng quinpirole on the interocular differences in RE (−1.42 ± 1.40 D after 2 weeks, and −1.87 ± 0.67 D after 4 weeks) and VCD (0.08 ± 0.01 mm after 4 weeks) were statistically significant (RE: P = 0.005; VCD: P = 0.028) compared to those of vehicle alone (ANOVA), but the effects of 10 and 1000 ng quinpirole, compared to those of vehicle, were not statistically significant in any comparison. Quinpirole also had no significant effect on anterior segment parameters at any dose tested (data not shown).

Thus, data from the quinpirole experiment (above) suggested that activation of the D2-like receptor family promoted the development of myopia in our albino guinea pigs; whereas, conversely, 2500 ng of the D2-like receptor antagonist, sulpiride, inhibited the development of myopia. After 4 weeks of sulpiride treatment, the interocular differences in RE and VCD were significantly different from those in the vehicle control group (RE, P = 0.003, Fig. 5A; VCD, P = 0.006, Fig. 5B, 1-way ANOVA).

In summary, the D1-like agonist SKF38393 and the D2-like antagonist sulpiride inhibited myopia development, but the D1-like antagonist SCH23390 and D2-like agonist quinpirole promoted myopia development. Figures 2 to 5 show the absolute RE and VCD and their differences between drug-treated and fellow eyes. The rather small difference in refraction that we observed after 4 weeks of treatment, only 1 to 2 D, may be the maximum effect that can be elicited after such treatment, in this particular strain of guinea pigs.
DISCUSSION

We have shown that progressive myopia, which developed in our albino guinea pigs without any experimental treatment, resembles experimentally-induced myopia in being affected by agents that mimic or interfere with dopaminergic signaling. However, the effect of apomorphine in these animals differed from its effect on form deprivation myopia in other animal models, including pigmented guinea pigs. Dopamine, a ubiquitous neuromodulator with a well-conserved cellular source in the retina, has long been implicated in the control of eye growth and prevention of myopia. 8,9 It exerts its effects through two groups of dopamine receptors: D1-like (D1 and D5) and D2-like (D2, D3, and D4). The biological actions of these two receptor families are complementary to one another; agonist binding to D1-like receptors activates adenylyl cyclase, but binding to D2-like receptors inhibits it. The actions of nonspecific agonists, such as dopamine itself and apomorphine, which bind efficiently to D1- and D2-like receptors, 32 may vary with agonist concentration and site of action.

Apomorphine has approximately a 10-fold higher affinity for D2-like than for D1-like receptors, and, thus, may affect them differentially at different concentrations. Therefore, apomorphine at the lowest effective dose may preferentially stimulate D2-like receptors, including the presynaptic D2 auto-receptors, which control the synthesis and release of endogenous dopamine. 33 However, at approximately a 10-fold higher dose, it could stimulate D1-like receptors, while D2-like receptors are saturated and unresponsive.

In our albino animals, apomorphine generated biphasic dose-response curves – inhibiting myopia, as expected, at a higher dose (250 ng per injection), but promoting it at a lower dose (25 ng per injection) – suggesting that it might inhibit myopia development via the lower-affinity D1-like receptors, but promote it via the higher-affinity D2-like receptors. The results of further tests, using receptor subtype-selective dopaminergic ligands, were consistent with this hypothesis; specifically, the presumed activation of dopamine D1-like receptors (by SKF38393) inhibited myopia development,
whereas the presumed activation of D2-like receptors (by quinpirole) promoted it. These results have been summarized, and a hypothesis to account for them is suggested in Figure 6. We proposed that the activation of D2-like receptors promotes myopia progression, whereas the activation of D1-like receptors inhibits it. Similar behavior has been reported in another rodent species by Zhou et al., who found that form-deprivation myopia was less in D2R-knockout mice than in wild-type mice.

**Comparison With Dopaminergic Effects in Form-Deprivation Myopia**

In pigmented guinea pigs, in contrast to albinos, we reported previously that experimentally induced (form-deprivation) myopia was only inhibited, in a dose-dependent manner, by daily subconjunctival injections of apomorphine (100% inhibition at 250 ng and ≥50% inhibition at 25 ng), whereas lower doses were ineffective and clearly did not promote myopia development. In the pigmented animals, subconjunctivally injected apomorphine was detectable in the vitreous, with a "dilution" factor ([quantity injected per eye]:[quantity recovered per vitreous body]) of approximately 10,000:1. 0.5 hours after injection, when the amount in the vitreous reached a maximum; the amount declined rapidly after that, but still remained quite constant (approximately 10% of the maximum) between 8 and 24 hours after injection. For the delivery of apomorphine to ocular tissues, by the shallower subconjunctival route used in this study, is not expected to differ significantly from delivery by the slightly deeper peribulbar route used in the present study. Therefore, daily peribulbar injection of 250 ng apomorphine (which caused maximal suppression of myopia in both studies) prevented form deprivation myopia in pigmented guinea pigs at vitreal concentrations of 4.4 nM (peak, at ≤0.5 hour) to ≤1 nM (sustained, at 8–24 hour), whereas 25 ng apomorphine (which caused maximal enhancement of myopia development in the present study) was effective at vitreal concentrations approximately 10-fold lower. Concentrations in other ocular tissues were not measured, but we can be sure that concentrations in the sclera were very much higher than those in the vitreous. In form-deprived monkeys, too, apomorphine (1%) administered topically in eye drops inhibited myopia development, and no enhancement of myopia was noted, but the site of action after administration in eye drops is completely unknown.

A similar antimonyia effect of dopaminergic agonists also has been reported in birds. Rohrer et al. found that intravitreal apomorphine inhibited (but never enhanced) form-deprivation myopia in young chickens, at a 50% effective dose (ED50) of 5 pg per day (producing a peak concentration in the vitreous humor = 108 PM), compared to an ED50 of 2.5 ng per day for subconjunctival injections according to Stone et al. This is a 50,000-fold reduction in drug amount from subconjunctival to vitreous in chicks, and a 5-fold excess over the 10,000-fold factor in guinea pigs, which might be expected, given the greater thickness of the (cartilaginous) sclera in the chick's eye. In the chick, [3H]spiperone (a D2-receptor agonist) reached average maximum retinal concentrations of 160 and 260 PM, whereas the maximum concentrations in the retinal pigment epithelium (likely containing some choroid) were 30 and 410 PM, respectively, in the first hour after intravitreal or subconjunctival ED50 doses. Spiperone concentrations in the sclera, after administering ED50 doses by the two routes, differed by approximately 4 × 10⁴ (0.4 PM vs. 1.7 nM, respectively). These data suggest that even in guinea pigs, with their much thinner sclera, peribulbar application is likely to deliver drugs to the outer coats of the eye (sclera and choroid) at concentrations several orders of magnitude higher than those delivered to retina and vitreous.

Very few previous studies have tested the effects of drugs specific for dopamine receptor subfamilies, except in chicks. Rohrer et al. observed that the antimonyia effect of intravitreal apomorphine in form-deprived chicks was blocked by large molar excesses of the D2-like antagonist spiperone, but not of the D1-like antagonist SCH23390, suggesting that intravitreal apomorphine inhibited myopia via D2-like receptors. However, they did not test receptor subtype-specific agonists. Schaeffel et al. observed in chicks that intravitreal sulpiride (a D2-like antagonist) promoted the development of FDM at 100 and 400 μg (ca. 1.2–4.7 mM, assuming vitreous volume = 250 μl), but inhibited it at 10 μg (ca. 120 μM), whereas the D1-like antagonist, SCH23390, inhibited FDM at 20 μg (ca. 280 μM), but tended to promote it at lower doses. Nickla et al. reported that intravitreal SKF-38395 (D1-like agonist; 10 nmol, approximately 2.55 μg) caused partial inhibition of negative lens-induced eye growth in chicks, and that SCH23390 (10 nmol, approximately 2.88 μg) blocked the vision-induced inhibition of lens-induced myopia; they observed no significant effect on the axial elongation of the eye.
Finally, McCarthy et al. found that intravitreal quinpirole (a D2-like agonist) inhibited the development of deprivation myopia at 10 nmol, or 2190 ng, a dose reported to influence retinal actions of dopamine released in the light, whereas SKF38393 had no effect at the same dose. Given that we lack comprehensive information about dopamine receptor pharmacology and localization in the eyes of birds, definitive interpretation of these data is impossible. Most authorities agree, however, that the release of dopamine in the retina prevents myopia, and that form-deprivation myopia is due to insufficient release of dopamine in the form-deprived retina. If this is so, then in the form-deprived (low retinal dopamine) condition, intraocular dopamine receptor antagonists ought to do little or nothing unless they act upon more distant (e.g., choroidal) targets, where the release of dopamine from local sources is regulated differently from release in the retina, or they act via lower-affinity (e.g., nondopaminergic) receptors in the retina or other readily accessible target tissues.

Are Guinea Pig Dopamine Receptors Idiosyncratic?

One possible explanation for the unexpected results reported in the present paper might be that the drugs we tested, which have been characterized mainly at human or mouse receptors, act differently at dopamine receptors in guinea pig than in other mammalian species. This seems not to be the case. In various guinea pig test systems: (1) apomorphine was found to exert D2-like actions in heart membranes, IC$_{50}$ = 0.8 μM (5), and not to act via sigma or α-adrenergic receptors; (2) SKF38393 acted as a D1-like agonist, displacing [125I]SCH23390 from substantia nigra or corpus striatum plasma membranes, IC$_{50}$ = ca. 20 nM, had no effect on quinpirole-induced locomotor activity, and were ineffective at α$_2$-adrenergic receptors; (3) quinpirole acted as a D2-like agonist, binding to heart cell membranes with IC$_{50}$ = 1.5 μM, hyperpolarizing neurons from substantia nigra at 10 μM, and inhibiting release of dopamine from retinal pieces in vitro, specifically via D2 receptor (and not D3 receptor) autoreceptors, but was ineffective at α$_2$-adrenergic receptors.

**Figure 4.** Effects of the D2-like receptor agonist, quinpirole, on the development of myopia. Format is as for Figure 2. Again, data are shown (A) for RE and (B) VCD as separate dose-response functions for quinpirole-treated and fellow control eyes, in the lower half (y-axis on left); and as bar graphs of interocular differences, treated minus control, in the upper half (y-axis on right), for each parameter. Vehicle alone caused small but statistically significant myopic shifts in RE and VCD (paired t-test); but again quinpirole enhanced myopia progression (RE and VCD), especially at the higher doses. Symbols and error bars are as for Figure 1.

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Dopaminergic Agents Effects on Myopia

FIGURE 5. Effects of the D2-like receptor antagonist, sulpiride, on the development of myopia. Format is as for Figure 3. Vehicle alone (containing DMSO, instead of water as in vehicle for all other drugs) did not cause myopic shifts in RE and VCD (paired t-test), but sulpiride inhibited myopia progression at the single dose tested. Symbols and error bars are as for Figure 1.

(4); (4) SCH23390 had no effect on abnormal, quinpirole-induced (D1-like receptor-specific) locomotor activity, but blocked dopamine transport from blood to brain by 77% at 0.5 μM45; and (5) sulpiride did inhibit quinpirole-induced locomotor activity, blocked quinpirole-induced hyperpolarization of isolated substantia nigra neurons, and bound to D2-like receptors in cardiac cell membranes with IC50 = 0.5 μM.36 Dopamine itself, at 5 to 100 μM, bound to α2-adrenoreceptors in isolated ideal synaptosomes and gastric cell membranes, as did sulpiride, but not spiperone. Apomorphine and sulpiride, at 10 μM, were found not to interact with sigma-receptor ligands at brain cell membranes.47 In the present studies we administered drugs by a peribulbar rather than intravitreal route, because the thin sclera of guinea pigs might not survive repeated intravitreal injections. Such differences in route of administration must cause differences in concentration gradients, and, therefore, affect signaling cascades differentially, in the different layers of the eye. Intraorbital delivery should give drugs access to varied and abundant targets that are unlikely to respond to intravitreally injected drug, such as the ciliary ganglion. The action of apomorphine, if any, at these targets will depend upon the types and densities of local receptors, and what barriers the drug must pass to reach them. Finally, not only DA receptors, but also adrenergic and serotonergic receptors may be responsive to some of these drugs at high concentrations.

In summary, despite important differences in animal models and routes of drug administration, the effects of DAs on eye growth reported here are similar to many of those reported previously by others, and these drugs are as specific for D1-like vs D2-like receptors in guinea pigs as they are in other mammals.

CONCLUSIONS

We suggest the following interpretation of our results:

1. In guinea pigs, dopamine receptors are affected locally (i.e., not systemically or at a distance) by drugs injected into the peribulbar space. This is supported by the absence of effects on the contralateral eye, or obvious systemic effects, such as changes in locomotion, eating habits, or coordination.

2. In our experiments, the drugs may have diffused through the thin, fibrous sclera (approximately 110 μm23 to act upon targets in the retina, pigment epithelium, and choroid, as well as the sclera itself; they also could be expected perhaps to act on other accessible structures within the orbit, such as the ciliary ganglion. The ciliary ganglion is unlikely to be the site of action of dopamine receptor ligands; however, even though ciliary ganglion cells have been reported to receive dopaminergic or adrenergic innervation in some mammals, because detectable stores of catecholamines or their synthetic enzymes have been sought, but not detected, in preganglionic terminals in the ciliary ganglion of the guinea pig.48–50 Therefore, we can assume that the effects of dopaminergic drugs observed in our experiments were mediated by dopamine receptors in the eye itself.

3. Agonists binding to the relatively low-affinity D1-like receptors (SKF38393, and apomorphine at relatively high concentration) inhibited myopia progression, whereas an antagonist at these receptors (SCH23390) promoted it. These results are consistent with the conclusions that ocular elongation and myopia are constitutive in the untreated eyes of these animals, and mean dopamine levels around the D1-like receptor targets of the injected drugs are intermediary. Therefore, supplementation of endogenous dopamine with exogenous D1-like agonists can further inhibit myopia progression, whereas antagonists remove excitation at D1-like receptors, thereby removing growth-restraint and permitting further development of myopia. Need-
less to say, perhaps, more complicated (e.g., parallel, polysynaptic) pathways and nondopaminergic mechanisms are likely also to be involved in the control of eye growth, but these are beyond the scope of the present investigation.

4. Conversely, agonists binding to the relatively high-affinity D2-like receptors (quinpirole, and possibly apomorphine at relatively low concentration) promoted myopia progression, whereas an antagonist at these receptors (sulpiride) inhibited it. Among possible explanations, for why the responses caused by D2-like receptor ligands are opposite to those caused by D1-like receptors, are: if the D2-like receptors were “postsynaptic” (on nondopaminergic cells), as many of them most certainly are, then the activation of D2-like receptors by an exogenous agonist (or endogenous dopamine) might inhibit the constitutive restraint of axial elongation and myopia-development by some downstream mechanism; or if the D2-like receptors were presynaptic (on dopaminergic cells), as they are also very likely to be, then activation of these autoreceptors by D2-like agonists may inhibit the synthesis and release of endogenous dopamine. In this way, the D2-like agonist would decrease endogenous dopaminergic activity; thus, either diminishing the dopaminergic activation of myopia-preventing mechanisms through D1-like receptors (above, point 3), or enhancing the activation of myopia-promoting mechanisms via “postsynaptic” D2-like receptors (above, point 4).

5. The actions of D2-selective agents are consistent with the conclusions drawn from the actions of D1-like agents; that is, that ocular elongation and myopia are constitutive processes in the untreated eyes of these animals; and that the constitutive levels of endogenous agonist (dopamine) are intermediate, so that added agonists and antagonists, respectively, can further promote and inhibit myopia progression.

6. While retinal dopamine levels are reduced dramatically in experimental myopia in chicks, in which form-deprivation inhibits retinal dopamine synthesis and release, retinal dopamine levels in our albino guinea pigs were not significantly different from those in pigmented ones (99.5 ± 51.6 vs. 89.9 ± 21.2 ng/mg retinal wet weight, not significant, n = 7); however, the choroidal dopamine level in albino guinea pigs was found to be significantly less than that in pigmented ones (31.97 ± 8.85 vs. 68.69 ± 19.75 ng/mg retinal wet weight, P = 0.02, n = 6; Jiang et al., 2013, unpublished studies). Dopamine D1 receptors have been identified in human uveoscleral tissue. Therefore, it seems possible that the ocular dopamine receptors in our albinos, especially in sclera or choroid, may be in a chronic state of partial excitation, which could be responsible for the opposite or complementary effects of receptor subtype-specific agonists and antagonists on eye growth in these animals.

These unexpected results cannot yet be definitively explained. Nevertheless, they are interesting and potentially important, because so little is known about the control of visual and ocular development in albinism, and because relevant questions are raised here about the target tissues and cells, molecular mechanisms, and specificity of action of the dopaminergic drugs that are commonly used in experimental myopia research.
Dopaminergic Agents Effects on Myopia

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References


