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Dopaminergic agonists that result in ocular growth inhibition also elicit transient increases in choroidal thickness in chicks

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ABSTRACT

The dopaminergic system has been implicated in ocular growth regulation in chicks and monkeys. In both, dopamine D2 agonists inhibit the development of myopia in response to form deprivation, and in chicks, to negative lenses as well. Because there is mounting evidence that the choroidal response to defocus plays a role in ocular growth regulation, we asked whether the effective agonists also elicit transient thickening of the choroid concomitant with the growth inhibition.

Negative lenses mounted on velcro rings were worn on one eye starting at age 8–12 days. Intravitreal injections (20 μl; dose = 10 nmole) of the agonist (dissolved in saline) or saline, were given through the superior temporal sclera using a 30G needle. Eyes were injected daily at noon, for 4 days, and the lenses immediately replaced. Agonists used were apomorphine (non-specific; $n = 17$), quinpirole (D2; $n = 10$), SKF-38393 (D1; $n = 9$), and saline controls ($n = 22$). For the antagonists, the same protocol was used, but on each day, the lenses were removed for 2 h. Immediately prior to lens-removal, the antagonist was injected (20 μl; dose = 5 nmole). Antagonists used were methylergonovine (non-specific; $n = 12$), spiperone (D2; $n = 20$), SCH-23390 (D1; $n = 6$) and saline controls ($n = 27$). Comparisons to saline (continuous lens wear) controls were from the agonist experiment. Axial dimensions were measured using high frequency A-scan ultrasonography at the start of lens wear, and on day 4 prior to the injections, and then again 3 h later. Refractive errors were measured using a Hartinger's refractometer at the end of the experiment.

Apomorphine and quinpirole inhibited the refractive response to the hyperopic defocus induced by the negative lenses (drug vs saline controls: −1.3 and 1.2 D vs −5.6 D; $p < 0.005$ for both). This effect was axial: both drugs prevented the excessive ocular elongation (change in axial length: 233 and 205 μm vs 417 μm; $p < 0.01$ for both). Both drugs were also associated with a transient thickening of the choroid over 3 h (41 and 32 μm vs −1 μm; $p < 0.01$; $p = 0.059$ respectively) that did not summate: choroids thinned significantly over the 4 day period in all lens-wearing eyes.

Two daily hours of unrestricted vision during negative lens wear normally prevents the development of myopia. Spiperone and SCH-23390 inhibited the ameliorating effects of periods of vision on lens-induced refractive error (−2.9 and −2.8 D vs 0.6 D; $p < 0.0001$), however, the effects on neither axial length nor choroidal thickness were significant. These data support a role for both D1 and D2 receptors in the ocular growth responses.

1. Introduction

Dopamine is a neurotransmitter found in a subset of amacrine cells in most vertebrates, and in interplexiform cells in some species (Kramer, 1971; Dowling and Ehinger, 1978; Witkovsky and Dearry, 1992). The functions attributed to dopamine are numerous, predominant among these is as a mediator of light-adaptive changes in retinal circuitry and in RPE physiology, including photoreceptor retinomotor movements, pigment dispersal in RPE cells, and horizontal cell uncoupling. It is also an integral component of the retinal circadian oscillators, functioning as the "day" signal in a mutually-inhibitory reciprocal relationship with the hormone melatonin (reviews: Witkovsky and Dearry, 1992; Witkovsky, 2004). Another potential function that may be unrelated to diurnal phenomena and light-adaptive mechanisms is as a signal molecule in the visual regulation of eye growth. In both chickens and monkeys, dopamine content decreases in retinas of fast growing eyes developing axial myopia in response to deprivation of form vision (Stone et al., 1989;

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Iuvone et al., 1991; Rohrer et al., 1993) or to hyperopic defocus induced by negative lens wear (Guo et al., 1995). Conversely, dopamine content increases in retinas of slow-growing eyes recovering from form deprivation myopia (Pendrak et al., 1997). Furthermore, intravitreal injections of the dopamine agonist apomorphine prevents the development of deprivation-induced myopia in both chicks and monkeys, and negative lens-induced myopia in chickens (Schmid and Wildsoet, 2004). More recent work showed that exogenous dopamine (Gao et al., 2006) and its precursor levodopa (Mao et al., 2010) have similar preventative effects on myopia development induced by form deprivation in rabbits and guinea pigs, respectively. Finally, the amount of myopia induced by varying amounts of image degradation is directly proportional to the reduction in retinal dopamine in chicks (Stone et al., 2006).

The finding that the D2 receptor agonist quinpirole, but not the D1 agonist SKF-38393 was effective in inhibiting form deprivation-induced myopia (McCarthy et al., 2007) argues for a D2 receptor-mediated mechanism. Accordingly, the D2 receptor antagonist sulpiride enhanced deprivation-induced myopia (Schaeffel et al., 1995). By the same token, when the D2 antagonist spiperone was co-administered with apomorphine, it attenuated the protective effect of apomorphine (Rohrer et al., 1993) and when injected prior to daily periods of unrestricted vision in form deprived chick eyes, it prevented the ameliorative effect of the vision on the development of myopia (McCarthy et al., 2007).

The purpose of the present study was to further examine the role of dopamine in the signal cascade mediating emmetropization. Specifically, we asked whether the growth inhibition effected by D2 receptor agonists in negative lens-wearing eyes was consistently associated with increases in choroid thickness, which would be expected if the choroidal response is part of the signal pathway leading to ocular growth inhibition, as has been suggested (Nickla, 2007). If this was true, it would follow that D1 agonists would not affect choroidal thickness. Furthermore, injections of a specific D2 antagonist should counter the growth inhibitory effects of daily periods of vision, and not be associated with choroidal thickening. We found that the effective growth inhibitors apomorphine (non-specific) and quinpirole (D2 agonist) both resulted in a transient choroidal thickening, while the relatively ineffective D1 agonist SKF-38393 did not. Contrary to expectation (McCarthy et al., 2007), we found that the D2 antagonist spiperone had only a partial effect in preventing the refractive inhibition normally induced by periods of vision in lens-wearing eyes; these eyes became less myopic than no-vision saline-injected lens-wearing eyes. The choroidal response was not affected. While these data are consistent with previous work indicating a role for D2 receptors, they also suggest involvement by the D1 receptor family as well. Some of these results have been presented in abstract form (Dhillon and Nickla, 2008). Part of the data in Fig. 1C has been published (Nickla and Wallman, 2010).

2. Methods

2.1. Subjects

Subjects were White Leghorn chickens (*Gallus gallus domesticus*), hatched in an on-site incubator and raised in temperature-controlled brooders. The light cycle was 12L/12D (8:00 am to 8:00 pm). Food and water were supplied ad libitum. In all experiments, the right eye was treated and the left eye served as the untreated controls. Care and use of the animals conformed to the ARVO Resolution for the Care and Use of Animals in Research.

2.2. Experimental design

2.2.1. Agonists

Negative lenses (−10 D) mounted on velcro rings were attached to the matching ring that was glued to the feathers around one eye, starting at age 8–12 days. There are no age-related differences in the responses to negative lens wear over this range of age (Wildsoet and Wallman, 1995). On each day for 4 days, chicks were anaesthetized with isoflurane inhalation anesthesia, and intravitreal injections (20 μl, for a dose of 10 nmoles injected) of the drug dissolved in saline, or saline (0.75%; $n = 22$) were given at approximately noon. Each experiment had a number of saline controls to control for inter-experiment variability; these data were combined, as there were no significant differences between experiments. Injections used a 30G needle, going through the skin of the lids over the superior temporal sclera after removing the feathers and cleaning the skin with alcohol. Care was taken to use the same injection site for subsequent injections. The needle remained in place for 30 s before being slowly withdrawn while the skin around the site was held tightly together using a small forceps. The lenses were replaced immediately. The agonists used (all Tocris Bioscience) were apomorphine (non-specific; $n = 17$), quinpirole ($n = 10$; D2/D4 selective: Sullivan et al., 1998), SKF-38393 ($n = 9$: K_i for D1 vs D2, D3, D4 receptors = 1.0 vs 150, 5000, 1000 nM; Seeman and Van Tol, 1994). The data from this group were also used in the analysis of the effects of the antagonists (Fig. 2).

2.2.2. Antagonists

For dopamine antagonists, the same protocol as above was used, however, on each day for 4 days the lenses were removed for a 2-h period starting at around noon. Immediately prior to lens removal, the following drugs were injected, in 10 μl, for a dose of 5 nmole: spiperone (Tocris; $n = 20$), a D2/D4 receptor antagonist (K_i for D2 vs D3, D4, D1, D5 = 0.06 vs 0.6, 0.08, 350, 3500; Seeman and Van Tol, 1994), SCH-23390 (Sigma; $n = 6$), a D1 receptor antagonist, methylergonovine maleate (Sigma; $n = 12$), a non-specific dopaminergic antagonist, or saline ($n = 27$) as injection controls. In graphs, “sal/vis” refers to the saline-injected group, in which the lenses were removed daily, like the drug-injected groups. The data from saline-injected eyes wearing lenses continually are from the agonist experiments described above, and are denoted “sal/lens” in graphs. For spiperone, the procedure used by Ashby et al. (personal communication; Ashby and Schaeffel, 2010) was followed. Spiperone was dissolved in a 1 mg/ml solution of ascorbic acid to yield 500 μM concentration, and heated to 30° for 10 min while stirring. Doses for all drugs were based on the results of McCarthy et al. (2007).

For all experiments, axial dimensions were measured using high frequency A-scan ultrasonography (details in Nickla et al., 1998) at the start of lens wear, and on day 4 immediately prior to the injections, and then again 3 h later. Refractive errors (RE) were measured using a Hartinger’s refractometer (details in Wallman and Adams, 1987) at the end of the experiment. Statistical analyses between groups used an ANOVA and post-hoc Dunnett adjustment.

3. Results

3.1. Dopamine agonists

We examined the effects of three dopaminergic agonists on the refractive responses to hyperopic defocus induced by negative lens wear (ANOVA, $p = 0.0001$; Fig. 1A). Both the non-specific agonist apomorphine and the D2 receptor agonist quinpirole significantly inhibited the development of myopia in response to negative lenses