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Effects of 7-Methylxanthine on Deprivation Myopia and Retinal Dopamine Release in Chickens

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Keywords
Myopia · Animal model · Biochemistry/pharmacology · 7-Methylxanthine · Dopamine

Abstract
Introduction: Intake of 7-methylxanthine (7-MX), an adenosine receptor (AR) antagonist, has been shown to inhibit school myopia in children and deprivation myopia in rhesus monkeys, but the underlying mechanisms are not known. Also retinal dopamine seems to be involved in the control of eye growth, and in the brain, ARs and dopamine receptors interact widely by heteromerization. We have studied whether 7-MX can inhibit deprivation myopia also in chickens and whether inhibition may involve the retinal dopamine system. ***Methods:*** 7-MX was applied by either tube-feeding (100 µg/g body weight, twice a day) or intravitreal injection (12.5 µg, every other day). Forty-eight 2-week-old chicks wore unilateral diffusers and were randomly assigned to either the tube-feeding group (involving 7-MX, vehicle [xanthan gum], or no feeding, for 13 days) or the intravitreal injection group (involving 7-MX, vehicle, or DMSO, for 8 days). Refractions (REs), ocular biometry (AL, VCD), and scleral and choroidal thickness (ChT) were measured before and after treatment. Dopamine and dihydroxyphenylacetic acid (DOPAC) content were determined in retina and vitreous by HPLC at the end of the experiments. ***Results:*** No matter how 7-MX was applied, it did not inhibit deprivation myopia in chicks. No significant differences were observed in RE, VCD, AL, and scleral fibrous layer thickness. Feeding 7-MX produced more choroidal thinning in the open contralateral eye compared to control eyes in the vehicle-fed group (–40 ± 14 vs. –1 ± 7 µm, unpaired *t* test, $p < 0.05$). DOPAC and dopamine concentration in vitreous and DOPAC concentration in retina did not change with 7-MX. Vitreal dopamine content was significantly decreased in deprived eyes in the groups fed with the vehicle xanthan gum (paired *t* test, $p < 0.01$) but not in 7-MX-treated eyes, perhaps indicating a small effect of 7-MX on dopamine. ***Conclusions:*** In our study, 7-MX had no effect on DM in chicks and only minor effects on ChT and retinal dopamine. It remains unclear whether 7-MX inhibits myopia through a retinal mechanism or whether it acts directly on choroid and sclera. In the latter case, the finding that myopia is suppressed in mammals but not birds might be explained by differences in scleral structure.

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Introduction

Myopia has become the most common ocular disorder due to an increase in prevalence in recent decades. In the USA, the prevalence of myopia increased from 25.0 to 41.6% between 1971 and 2004 [1]. During the same time period, prevalence in the UK among approximately 11-year-old children increased from 4.0 [2] to 11.9% [3]. The situation is even more alerting in East Asia, with a prevalence of nearly 88% in 2015 in some parts of eastern China [4]. Myopia can be corrected by spectacle wear or surgery, but higher myopia (-6D or more) is associated with an increased risk of cataract, glaucoma, and myopic macular degeneration [5, 6]. Therefore, effective measures to prevent and control myopia are urgently needed.

Myopia is characterized by an increase in eye size and is accompanied by structural changes in the sclera, the outer coat of the globe that determines ocular shape. The currently available treatments for myopia include multifocal soft contact lenses, radial refractive gradient lenses, bifocal spectacles, or orthokeratology [7]. In terms of pharmaceutical interventions, atropine has recently received most attention [8, 9]. Major disadvantages of topical atropine are its severe side effects at higher doses (1% eye drops) and the fact that low concentration of atropine can only be used off-label in most countries [10]. Another potential agent to reduce myopia progression is 7-methylxanthine (7-MX). 7-MX, a metabolite of caffeine, is a nonselective adenosine receptor (AR) antagonist. It has been demonstrated that 7-MX administration reduces myopia progression in children [11], rabbits [12], guinea pigs [13], and macaques [14] after oral administration. In the chicken model of myopia, 7-MX has only been investigated in one study [15], which suggested a weak protective effect of 7-MX on lens-induced myopia but no effect on deprivation myopia (DM) [16]. Although the specific site of action of 7-MX remains unknown, the sclera appears to be the most probable target tissue after it had been shown that 7-MX prevented scleral thinning reduction of scleral collagen fibril diameters in guinea pigs when myopia was induced [13]. Similar observations were made in pigmented rabbits [12]. Structural changes in the sclera could prevent axial elongation, a key feature of progressing myopia. Another possible target tissue is the choroid as it was found that oral 7-MX thickened the choroid in macaques [14]. It is unknown if 7-MX can cross the blood – retina barrier that is represented by the retinal pigment epithelium (RPE) and the pecten in the chicken [17]. Circulating caffeine can readily cross the blood – brain barrier [18]. It is also possible that orally administered 7-MX can enter the retina although this was never demonstrated.

ARs are expressed in retinal arterioles and perivascular retinal tissue [19]. If 7-MX would reach the retina, it could affect retinal blood flow. To date, 4 AR subtypes, that is, A1AR, A_{2A}AR, A_{2B}AR, and A3AR, have been identified and all are expressed in the posterior ocular walls including retina, choroid, and sclera of guinea pigs [20], rhesus monkeys [21], and also in human scleral fibroblasts [22]. Similarly, all 4 AR subtypes are expressed in the retina of rats [23]. In chicks, A1AR and A_{2A}AR were found in the retina [24]. There is experimental evidence that ARs may play an important role in myopia development. A_{2A}AR knock-out mice showed relative myopia and a higher density of scleral collagen fibrils although their diameter was reduced, compared with wild-type mice [25]. In addition, decreased A1AR and increased A_{2B}AR protein expressions were found in the retina of guinea pigs developing deprivation myopia [20]. Moreover, a single nucleotide polymorphism detected in the A_{2A}AR exons was proposed to be related to the high myopia in a Chinese population [26]. It is still unknown which of the AR subtype(s) may be involved in the inhibition of myopia by 7-MX.

Retinal dopamine release is known to be reduced during the development of experimentally induced myopia [27, 28]. There are 2 major subtypes of dopamine receptors (DRs): D1-like receptors (D1R and D5R) and D2-like receptors (D2R, D3R, and D4R). Although initial experiments seemed to suggest that stimulation of the D2-like DRs caused inhibition of myopia, later studies also supported a role of D1 receptors [27, 28]. In the brain, AR and DR interact widely by AR-DR heteromerization, forming mainly A1R-D1R and A_{2A}R-D2R heteromers. In behavioral and biochemical studies, adenosine – dopamine interactions were found to be antagonistic [29]. Thus, nonselective AR antagonists can boost the activity of DRs [30]. One of the explanations for antagonistic interactions may be that the A1R activation within the heteromer changes the binding of dopamine to D1 receptors [31]. Similarly, A_{2A}R-D2Rs also display antagonistic interactions [32, 33]. As a key modulator of striatal neuronal activity, the balance between A_{2A}R and D2R is essential for the function of heteromers.

In the current study, we have studied the effects of 7-MX on the development of deprivation myopia in chickens. Both oral and intravitreal application routes were tested to narrow down possible target sites. Changes in refraction (RE), axial length, and choroidal and scleral

thickness were measured. In addition, based on studies showing adenosine–DR interactions, we have analyzed the effects of 7-MX on retinal dopamine release.

Methods and Materials

Animals

All experiments adhered to the statement of the Association for Research in Vision and Ophthalmology (ARVO) for the Use of Animals in Ophthalmic and Vision Research. Procedures were approved by the Commission for Animal Welfare of the Medical Faculty of the University of Tuebingen. One-day-old white leghorn chicks were obtained from a local hatchery (Weiss, Kirchberg, Germany) and were reared in large cages with water and food supplied ad libitum. The temperature and humidity were controlled in a suitable range, and the light cycle was 12:12 h light/dark.

Treatments

Tube-Feeding

The dosage of 7-MX used to inhibit deprivation myopia was based on published findings in macaques (100 mg/kg body weight, twice a day), guinea pigs (300 mg/kg body weight, once per day), and chickens (30 mg/kg body weight, twice a day) [16]. Since the reported dosage in chicks was rather low and the observed effects remained small, 7-MX in the current study was fed at a dosage of 100 mg/kg body weight (twice a day). We did not observe any side-effects of 7-MX. Chickens exhibited no abnormal behavior and gained weight normally during the whole duration of the treatment with 7-MX or the vehicle (xanthan gum) compared to the control group (63.51 ± 7.185 vs. 58.20 ± 6.237 and 67.64 ± 3.911 g, respectively).

7-MX was kindly provided by MD Klaus Trier (Hellerup, Denmark). Xanthan gum (Sigma, St. Louis, MO, USA; 0.3%, dissolved in saline) was used to suspend 7-MX uniformly. Twenty-five 11-day-old chicks were monocularly deprived with diffusers and randomly assigned to 1 of 3 experimental groups: 7-MX group (n = 10), vehicle (xanthan gum) group (n = 10), and control group (n = 5). Chicks in 7-MX group were fed with the 7-MX at a dosage of 100 mg/kg of body weight, while those in the vehicle control group received the corresponding dose of xanthan gum twice a day (at 10 a.m. and 4 p.m.). The chicks in the control group were exempt from tube-feeding. Treatment lasted for 13 days.

Intravitreal Injection

As 7-MX has only low solubility in water and saline, it was dissolved in dimethyl sulfoxide (DMSO). Afterwards, it was further diluted with saline to a final concentration of 0.1% 7-MX in 10% DMSO. Experiment started when the chicks were 2 weeks old. Twenty-three chicks were monocularly deprived and randomly assigned to either 7-MX (n = 12) or DMSO treatment (n = 11). Chicks in the 7-MX group were binocularly intravitreally injected with 12.5 µL 0.1% 7-MX (corresponding to 12.5 µg), while those in the DMSO group were injected with the same volume containing only 10% DMSO. There were no reported doses of 7-MX for intravitreal injections. We chose the highest amount of 7-MX that could be dissolved in 10% DMSO. Injections were performed once every other day between 8:00 and 10:00 a.m. and continued for 8 days.

Measurement of Refraction (RE) and Axial Length (AL)

Ocular parameters were measured before and at the end of the treatment. An automated version of infrared photoretinoscopy was used to measure RE [34]. Ocular dimensions of normal and deprived eyes were examined by A-scan ultrasound as previously described [35]. Optical coherence tomography (OCT; Spectralis OCT, Heidelberg Engineering, Germany) was used to measure choroidal and scleral thickness. Since choroidal thickness (ChT) shows a diurnal rhythm [36], all OCT images were collected between 10:00 and 12:00. Care was taken to capture OCT images from the center of the fundus. The ChT was measured as the distance from the RPE layer to the outer boundary of the choroid as described recently [37]. There is a visible line between the scleral fibrous layer and cartilaginous layer in B-scan OCT images in chicks [38], and the public software package ImageJ was used to manually determine the thickness of the cartilaginous and fibrous sclera.

Sample Preparation and Measurement of Biogenic Amines by HPLC-ED

Animals were killed by inhalation of an overdose of diethyl ether. Eyeballs were enucleated immediately and cut with a razor blade perpendicular to the anterior–posterior axis, approximately 1 mm posterior to the ora serrata. The anterior segment was discarded. The vitreous was removed and quickly frozen in liquid nitrogen. One 8-mm tissue sample was taken from the posterior eye cup using a biopsy punch. Retina and choroid were isolated under a dissecting microscope, while the RPE cells were discarded. Tissues were frozen in liquid nitrogen and stored at –80 °C for subsequent high-performance liquid chromatography (HPLC) analysis.

All vitreous samples were weighed and homogenized in 750 µL mobile phase (Thermo Fisher Scientific, CA, USA) using a tissue lyser and 5-mm stainless steel beads (TissueLyser LT, Qiagen, Hilden, Germany) at 50 Hz for 4 min. For retinal and choroidal samples, 350 µL mobile phase was added before the homogenization. Twenty-five microliters of retinal and choroidal homogenate was saved for later protein determination (Pierce BCA Protein kit, Thermo Scientific, Rockford, IL, USA). All homogenized samples were centrifuged at 4 °C for 10 min at 14,000 g. The supernatant was filtered through a 0.2-µm nylon membrane filter (Thermo Scientific, Rockwood, MI, USA), and 25 µL was directly injected into the HPLC system. Samples were analyzed for catecholamine and indolamine content by HPLC (Ultimate 3000 LC with electrochemical detection ECD3000RS, Thermo Fischer Scientific) with coulometric detection as previously described [39]. In brief, a hypersil C18 column was used (150 × 3 mm, 3 µm) together with a test mobile phase (Thermo Fischer Scientific) containing 10% acetonitrile and 1% phosphate buffer. The flow rate was 0.4 mL/min and the potential at the first and second electrode was set to +370 and –200 mV, respectively. Dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid [40] concentrations were determined with a high reproducibility (98%). In retina and choroid, biogenic amine content was determined as nanogram per milligram protein (ng/mg protein), whereas in the vitreous, the amount of the substances was determined relative to wet weight (ng/0.1 g wet weight). As described in previous studies, vitreal DOPAC levels are considered a sensitive measure of DA release from the retina [41–43]. Recently, it has been demonstrated that also vitreal HVA content

is a good measure of dopamine turnover, since HVA content in the vitreous was significantly correlated with the amount of vitreal DOPAC [39].

Statistics

All data are reported as mean ± SEM. Two-tailed paired *t* tests were used to compare diffuser-treated eyes with their untreated contralateral eyes. The effect size (difference between diffuser-treated eyes and their untreated contralateral eyes) in the different treatment groups was analyzed by two-tailed unpaired *t* test with Bonferroni correction. A two-way analyses of variance (ANOVA) was used to analyze the impact of drug treatment and diffuser wear and possible interactions of both factors. Post hoc pairwise multiple comparisons were made using Tukey Kramer honestly significant difference test. Statistical tests were performed using jmp version 11 software (SAS institute). The limit of significance was set to $p < 0.05$.

Results

The baseline measurements of all ocular parameters showed no differences between treatment groups, both in the tube-feeding experiment ($p = 0.05$, one-way ANOVA) and in the intravitreal injection experiment ($p = 0.05$, unpaired *t* test). There was also no difference between both eyes at baseline ($p = 0.05$, paired *t* test).

Results of Tube-Feeding of 7-MX

At the end of the treatment, changes in refraction relative to baseline levels in the deprived eyes of the 7-MX-fed, vehicle-fed, and control groups were –5.83 ± 1.20 D, –3.80 ± 0.85 D, and –4.61 ± 1.37 D ($p = 0.05$), respectively (Fig. 1a). REs in the deprived eyes were highly significantly different from the REs in open control eyes. There were no significant differences between 7-MX-fed, vehicle-fed, and control groups, respectively. Furthermore, vitreous chamber depths and axial lengths were significantly increased in all deprived eyes, with no differences between 7-MX-fed, vehicle-fed, and control groups (Fig. 1b, c), showing that 7-MX at 100 mg/kg body weight twice a day had no effect on the development of deprivation myopia. There were also no differences in REs, vitreous chamber depths (VCD), and axial lengths between control eyes in the 3 groups, showing that 7-MX had also no effect on normal refractive development and eye growth in chickens (unpaired *t* test with Bonferroni correction, $p > 0.4$ for all parameters).

A two-way ANOVA, comparing differences in ChT during the treatment period, revealed that ChT was significantly changed by diffuser treatment (deprivation vs. untreated, $p = 0.014$) as well as by the drug treatment (7-MX vs. vehicle vs. control, $p = 0.036$). There was no significant interaction between both factors ($p = 0.49$). Eyes covered with diffusers displayed a significant thinning of the choroid, both in 7-MX-fed and in vehicle-fed groups (–65 ± 16 vs. –40 ± 14 µm and –48 ± 13 vs. –1 ± 7 µm

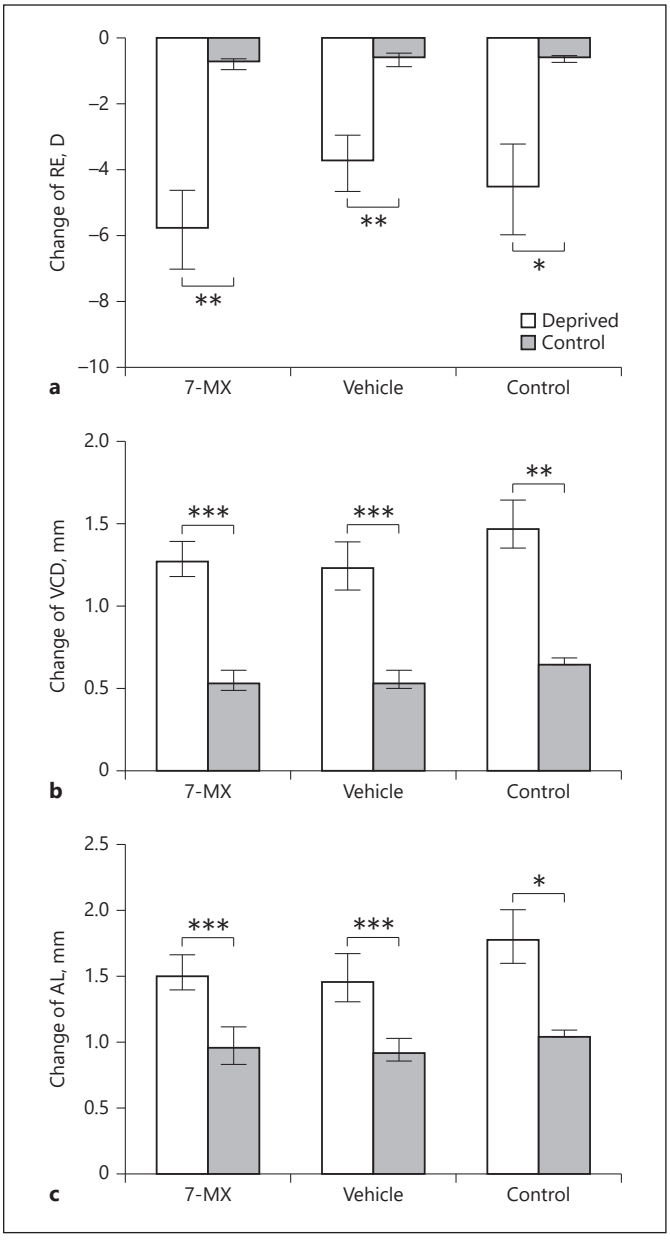

Fig. 1. Changes in RE (**a**), VCD (**b**), and AL (**c**) in the 3 groups of 7-MX-fed, vehicle-fed, and control chickens, after 13 days of treatment. All eyes covered with diffusers became more myopic and longer, with no difference whether they belonged to 7-MX-fed, vehicle-fed, or control chickens. Graphs show mean data ± SEM. Paired *t* tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. RE, refraction; 7-MX, 7-methylxanthine.

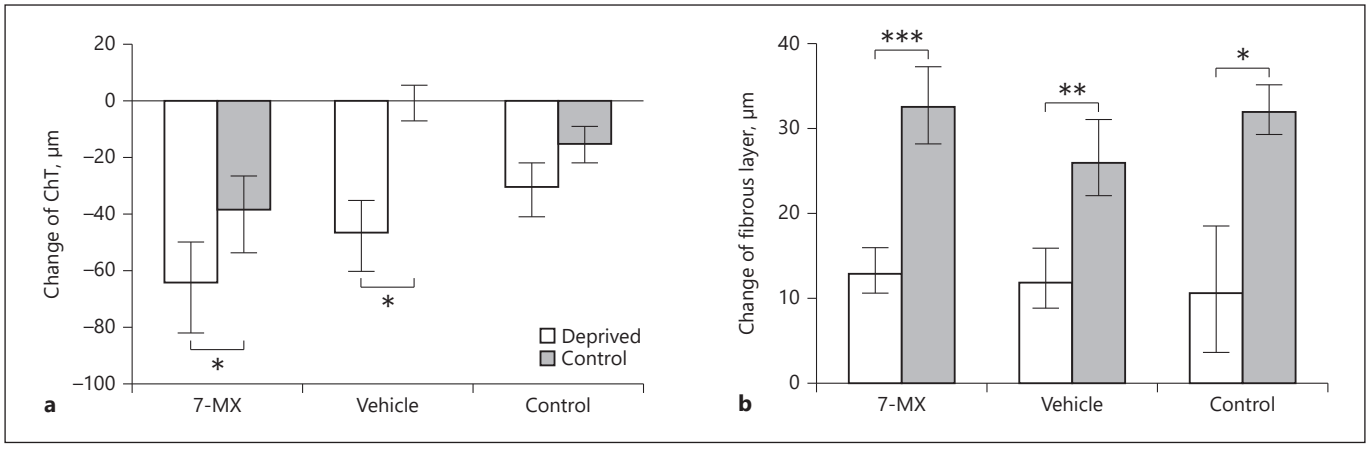

Fig. 2. Changes in ChT (**a**) and scleral fibrous layer thickness (**b**) in 7-MX-fed, vehicle-fed, and control chicks during the 13 days of treatment. **a** ChT declined both in deprived and in open eyes but more so in open eyes when 7-MX was fed. **b** Scleral fibrous layer remained thinner with diffusers in front of the eye, but there was no difference between 7-MX-, vehicle-fed, and no treatment groups. Graphs show mean data ± SEM. Paired *t* tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. ChT, choroidal thickness; 7-MX, 7-methylxanthine.

paired *t* test; Fig. 2a). In the control group, the interocular difference between deprived and open eyes was not statistically significant (change in ChT: –32 ± 10 vs. –16 ± 6 µm, $p = 0.25$). A comparison of deprived eyes in the 7-MX-fed, vehicle-fed, and control groups revealed no significant differences in the changes of ChT: 7-MX versus vehicle versus control group: –65 ± 16 vs. –48 ± 13 vs. –32 ± 10 µm (unpaired *t* tests, $p = 0.05$). In 7-MX-fed chicks, the choroid thinned more than in vehicle-fed chicks (–40 ± 14 µm vs. –1 ± 7 µm, unpaired *t* test, $p < 0.05$).

The fibrous layer became thicker in all eyes during the treatment period but significantly less so in diffuser-treated eyes (deprived vs. open eyes: 7-MX group: 13 ± 3 vs. 33 ± 4 µm, $p < 0.001$; vehicle-fed group: 12 ± 4 vs. 27 ± 5 µm, $p < 0.01$; control group: 11 ± 7 vs. 32 ± 3 µm, $p < 0.05$, respectively; Fig. 2b). The cartilaginous layer increased in thickness by about 11.5 µm over the treatment period but was unaffected by diffuser wear or drug treatment.

Two-way ANOVAs, comparing the amount of dopamine, DOPAC, and HVA in retina and vitreous, showed a highly significant effect ($p < 0.001$) of diffuser treatment on all metabolites. Only retinal dopamine and retinal HVA content were not changed (Fig. 3d, f). In line with previous studies, DOPAC content in vitreous and retina was significantly lower in eyes covered with diffusers, both in 7-MX- and vehicle-fed chickens (vitreous: 4.14 ± 0.59 vs. 7.16 ± 1.07 ng/100 mg wet weight and 3.62 ± 0.32 vs. 6.86 ± 0.85 ng/100 mg wet weight; retina: 0.86 ± 0.14 vs. 1.43 ± 0.16 ng/mg protein and 0.88 ± 0.14 vs. 1.53 ± 0.21 ng/mg protein). Also vitreal HVA content was significantly reduced in deprived eyes of 7-MX-fed and vehicle-fed chickens (3.51 ± 0.27 vs. 6.14 ± 0.25 and 3.49 ± 0.29 vs. 6.40 ± 0.49 ng HVA/100 mg wet weight). Vitreal dopamine content was changed by the diffusers only in the vehicle-fed chickens (0.27 ± 0.05 vs. 0.62 ± 0.09 ng/100 mg wet weight; Fig. 3b). There was no significant effect of 7-MX on catecholamine content in deprived eyes, neither in vitreous nor in retina.

Results of Intravitreal Injections of 7-MX

Covering eyes with diffusers induced myopia and axial eye growth in both 7-MX-injected and vehicle-injected eyes (Fig. 4a–c, paired *t* test, $p < 0.001$ for all comparisons). 7-MX had no suppressive effect on deprivation myopia (RE: –4.15 ± 0.63 vs. –5.62 ± 0.53 D, $p > 0.05$; VCD: 0.77 ± 0.04 vs. 0.84 ± 0.06 mm, $p > 0.05$; AL: 1.00 ± 0.07 vs. 1.08 ± 0.09 mm, $p > 0.05$). 7-MX injections also had no effect on refractive development in fellow eyes (RE: –0.46 ± 0.13 vs. –0.47 ± 0.11 D, $p > 0.05$; VCD: 0.28 ± 0.04 vs. 0.31 ± 0.03 mm, $p > 0.05$; AL: 0.61 ± 0.07 vs. 0.68 ± 0.007, $p > 0.05$).

Effects of diffuser wear and intravitreal 7-MX injections on choroidal and fibrous layer thickness changes are shown in Figure 5. While ChT remained unchanged in open eyes during the 8 days of treatment, diffusers over the eyes induced extensive choroidal thinning of about 80 µm in both 7-MX- and vehicle-injected eyes (ChT: –81 ± 5 vs. –77 ± 7 µm, $p > 0.05$; Fig. 5a). In agreement with a previous study [44], the fibrous layer became thinner in

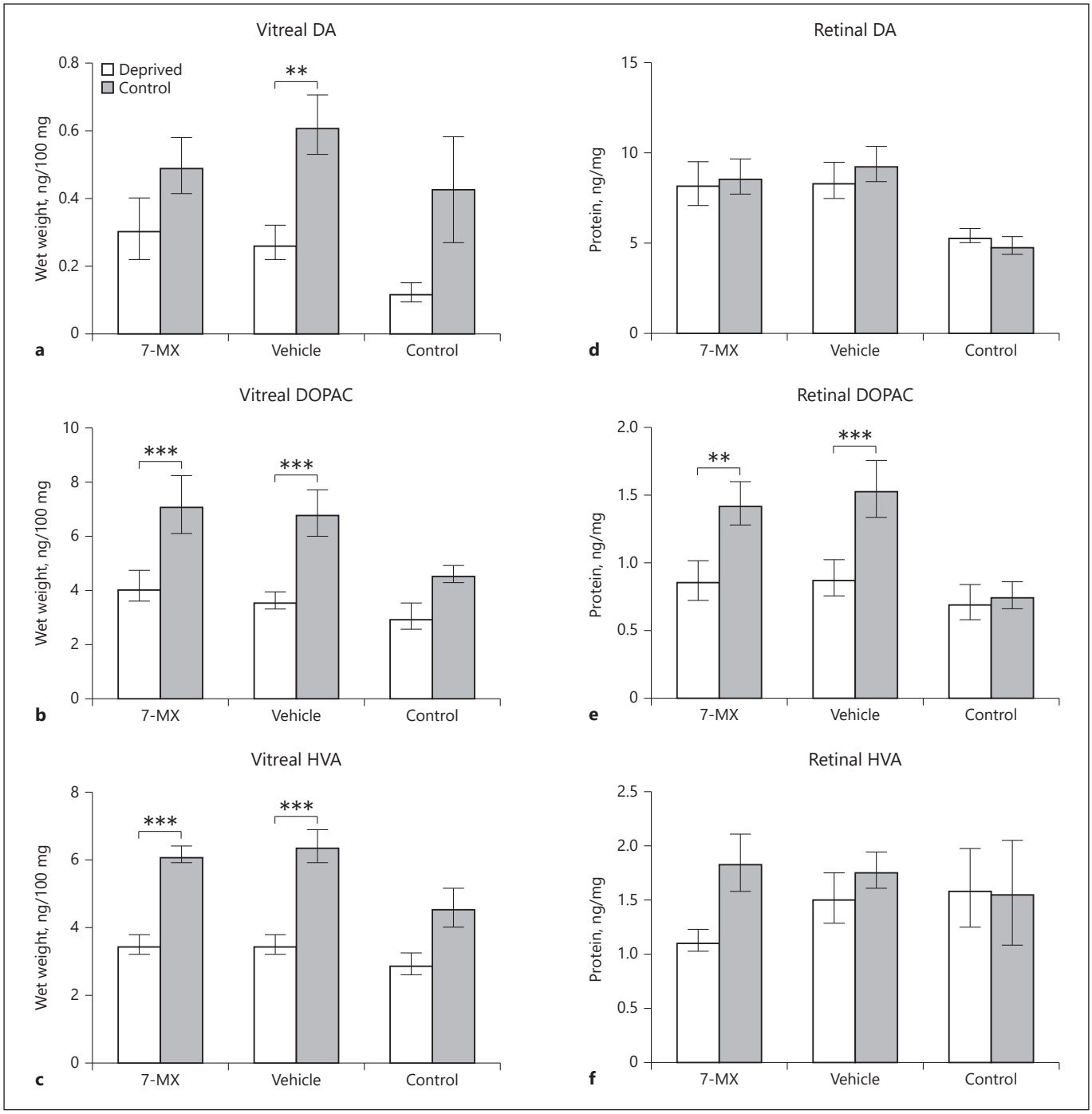

Fig. 3. Concentrations of DA, DOPAC, and HVA in vitreous and retina in 7-MX-fed, vehicle-fed, and control chickens after 13 days of treatment. Covering eyes with diffusers reduced DA, DOPAC, and HVA in the vitreous (**a–c**), while significant changes in the retina were confined to DOPAC (**e**). There were no clear trends for 7-MX application. Graphs show mean data ± SEM. Paired *t* test: ** $p < 0.01$; *** $p < 0.001$. 7-MX, 7-methylxanthine; DOPAC, dihydroxyphenylacetic acid.

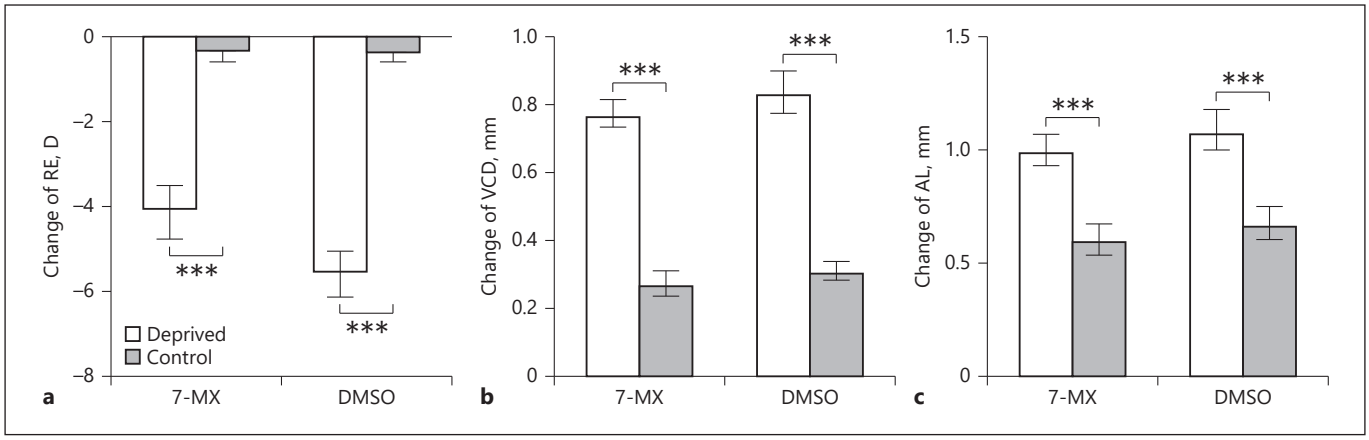

Fig. 4. Average change of RE (**a**), VCD (**b**), and AL (**c**) in 7-MX-injected and vehicle-injected control chicks after 8 days of treatment. Significant myopia was induced by the diffusers in both 7-MX- and DMSO-injected eyes, with no significant differences between both treatments. The graphs show mean data ± SEM. Paired *t* tests: *** $p < 0.001$. RE, refraction; 7-MX, 7-methylxanthine; DOPAC, dihydroxyphenylacetic acid.

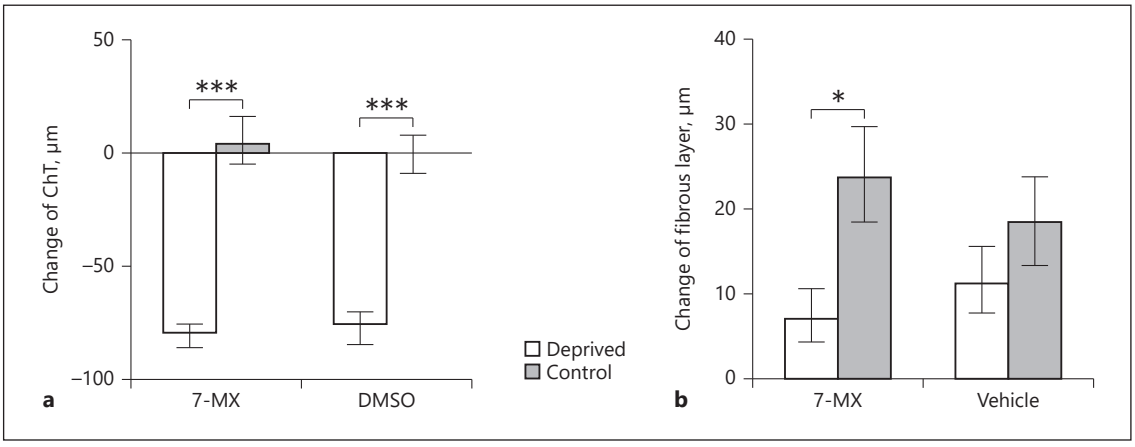

Fig. 5. Changes in ChT (**a**) and scleral fibrous layer thickness (**b**) in the 2 groups of 7-MX-injected and vehicle-injected control chicks after 8 days of treatment. Covering the eyes with diffusers caused severe thinning of the choroid and the fibrous layer, but 7-MX had only minor effects on these changes. The graphs show mean data ± SEM. Paired *t* tests: * $p < 0.05$; *** $p < 0.001$. ChT, choroidal thickness; 7-MX, 7-methylxanthine; DOPAC, dihydroxyphenylacetic acid.

myopic eyes compared to their open fellow eyes, although this difference was statistically significant only in the 7-MX-injected group (change in fibrous layer thickness: +8 ± 3 vs. +24 ± 6 µm, $p < 0.05$ in 7-MX-injected eyes, +12 ± 4 µm vs. +19 ± 5 µm, $p > 0.05$ in the DMSO-injected eyes). Neither the thickness changes in the choroid nor those in the fibrous sclera were significantly affected by intravitreal 7-MX injections. Thickness of the cartilaginous layer increased more in the deprived eyes of the 7-MX-injected eyes compared to the contralateral control eyes (7-MX vs. control: 18 ± 3 vs. 3 ± 3 µm, $p < 0.05$, DMSO vs. control: 13 ± 4 vs. 6 ± 2 mm, $p > 0.05$).

Intravitreal 7-MX injections did not prevent the decline of DOPAC in vitreous and retina in eyes covered with diffusers. As shown in Figure 6b and e, DOPAC content was significantly lower in deprived eyes compared to contralateral control eyes in both treatment groups (p 0.001 in vitreous and retina of both groups), but there was no difference between the 7-MX- and vehicle-injected eyes (vitreous: 2.76 ± 0.12 vs. 2.74 ± 0.14 ng/100 mg wet weight, p 0.05; Fig. 6a; retina: 0.54 ± 0.06 vs. 0.48 ± 0.04 ng/mg protein, p 0.05; Fig. 6d). Changes in HVA content were similar to those of DOPAC in both vitreous and retina (Fig. 6c, f). Vitreal dopamine content

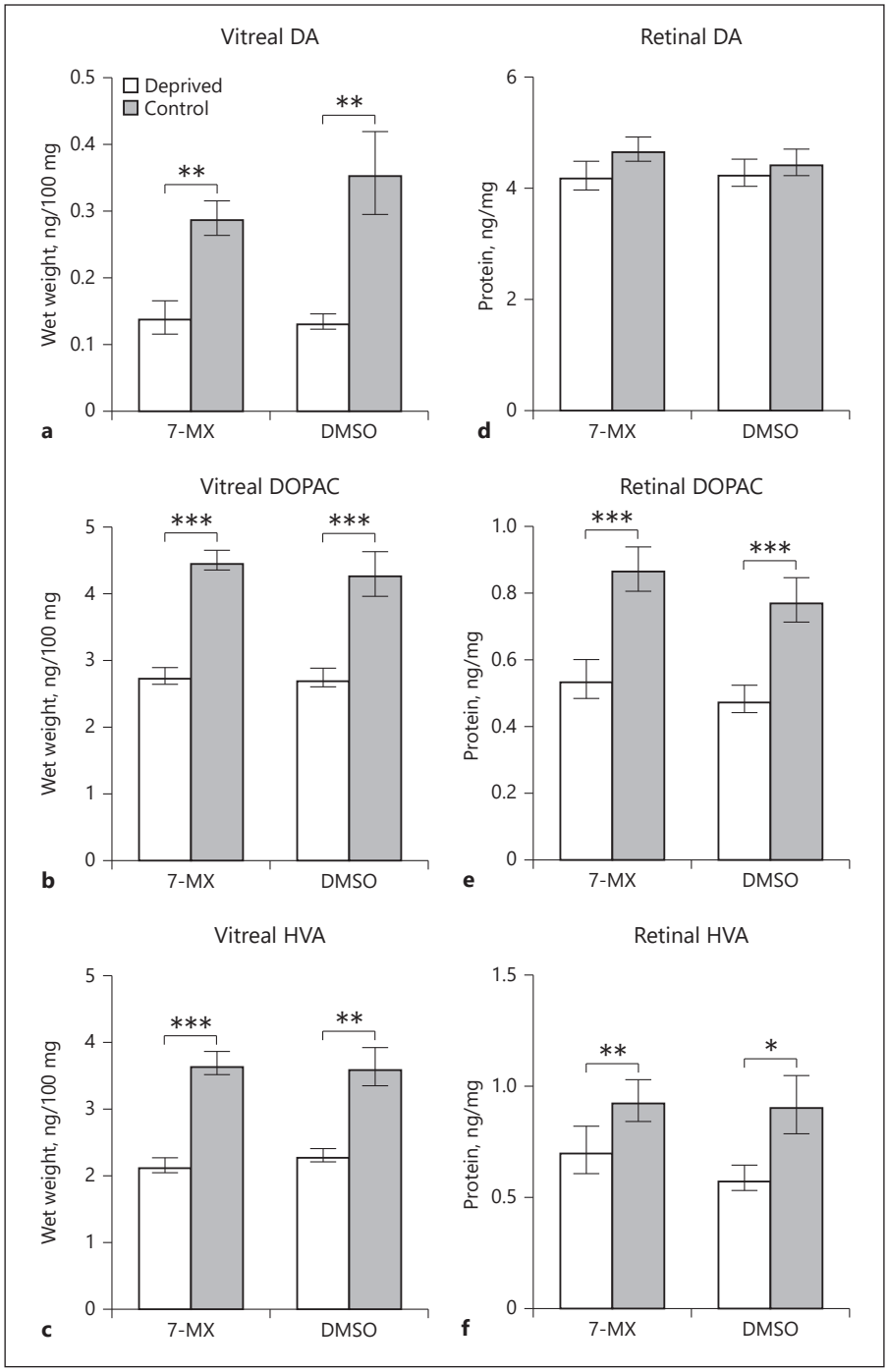

Fig. 6. Concentrations of DA, DOPAC, and HVA in vitreous and retina of 7-MX-injected and vehicle-injected eyes after 8 days of diffuser treatment. Covering the eye with diffusers reduced DA, DOPAC, and HVA concentrations in vitreous severely (**a–c**), but only DOPAC and HVA were lowered in the retina (**d–f**). Graphs show mean data ± SEM. Paired *t* tests: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. 7-MX, 7-methylxanthine; DOPAC, dihydroxyphenylacetic acid.

was significantly lower in deprived eyes in the DMSO group (0.14 ± 0.01 vs. 0.36 ± 0.06 ng/100 mg wet weight, p 0.01) and also in the 7-MX group (0.14 ± 0.03 vs. 0.29 ± 0.03, p 0.05; Fig. 6b). Retinal dopamine content was almost identical in deprived and open eyes in both groups (Fig. 6e).

Discussion

Morphological and biochemical changes that were observed in eyes covered with diffusers matched published results from our group and other labs. Diffusers induced the development of deprivation myopia with increased axial length and vitreous chamber depth. In addition, the

choroid and fibrous layer were significantly thinner in deprived eyes compared to untreated contralateral eyes, and the amount of vitreal DA, DOPAC, and HVA was reduced in myopic eyes.

Different from recently published studies in mammals [13, 14], 7-MX did not inhibit the development of deprivation myopia in chicks and had no effect on axial eye growth or vitreous chamber depth, neither after feeding nor after intravitreal injections. The result thereby fits to a small study in chicks in which also no protective effect of 7-MX on the development of experimentally induced deprivation myopia was found [16]. In this study, a 3 times lower amount of 7-MX was used for feeding the chicks. In the current study, we used a dose that was similar to the one that successfully suppressed myopia in mammalian myopia models. In addition to oral intake, we also delivered 7-MX by intravitreal injections but neither the higher dose nor the intravitreal injection route improved the effects of 7-MX on myopia in chicks. Oral intake of 7-MX also had no effect on refractive development and axial eye growth in open fellow eyes.

In monkeys, the choroid was found to thicken after 7-MX intake in both minus lens treated and fellow eyes [14], whereas, in our study, feeding of 7-MX had little effect on ChT – if at all, it induced more thinning in the uncovered control eyes. This result suggests a general effect of 7-MX on the choroid independent of deprivation. Several studies showed that the choroid, a highly vascularized tissue, might play an important role in emmetropization. ChT changes can be induced by treatment with spectacle lenses or diffusers and occur prior to growth changes of the sclera. To some extent, the changes in ChT can partly compensate for the imposed refractive errors, at least in chickens [45–48]. Since a thinner choroid is normally associated with the development of myopia, the small effect of 7-MX on the choroid could even suggest that it could accelerate eye growth in chicks. In published studies, the effects of the AR antagonists 7-MX and caffeine on ChT are controversial. In contrast to the thickening of the choroid observed in monkeys after 7-MX intake, a thinned choroid was observed in humans from 30 min to 4 h after oral intake of caffeine [49], another AR antagonist.

Many changes in scleral extracellular matrix synthesis and turnover occur during visually induced refractive errors and were studied in a variety of animal models. The sclera was proposed to be the most probable target tissue of 7-MX. Although no changes in AR protein expression were found in the choroid and sclera of deprived guinea pigs, feeding of 7-MX prevented the experimentally induced thinning of the scleral and the collagen fibril diameters in the posterior pole of the globe [20]. Also a recent study in pigmented rabbits found significant changes in collagen fibril diameter in the posterior sclera, although this effect was only significant when the deprived eyes of the vehicle-injected group and the control eyes of the 7-MX-fed group were compared [50]. An older study in rabbits showed that there were regional differences in the effect of 7-MX on anterior and posterior sclera, regarding both collagen concentration and fibril diameter [12]. Different from the results in guinea pigs and rabbits, we found that 7-MX had no effect on scleral fibrous layer thickness in chickens. However, given that there are structural differences in bird and mammalian scleras, this result may not be surprising. In most vertebrates, including chicks, the sclera is composed of 2 layers – an inner cartilaginous layer and an outer fibrous layer [51]. The sclera in humans and other mammals (i.e., marmosets, tree shrews, guinea pigs, and monkeys) is composed of only a fibrous layer. The lack of an effect of 7-MX on myopia development in chicks could therefore be due to the structural differences in the sclera – assuming that the sclera is the main target of 7-MX. In addition, there might be differences in AR amount and/or composition of different types of ARs in the different species, but to our knowledge AR expression in choroid and sclera of chicks was never investigated.

That there were no or only minor changes found in the retinal catecholamine system after 7-MX application supports the view that the targets of 7-MX are not in the retina. Since adenosine A1AR and A_{2A}AR are expressed in the chicken retina [24] and adenosine/dopamine interactions are well known from the central nervous system, it was expected that 7-MX should interact with retinal dopamine metabolism. However, the only minor effect was that vitreal dopamine content was significantly decreased in deprived eyes in chicks fed with the vehicle xanthan gum (paired t test, $p < 0.01$) but not in chicks fed with 7-MX. Furthermore, it is still not known whether 7-MX can cross the blood–retina barrier and reach targets in the retina. Therefore, we also tested the effects of 7-MX after intravitreal injection. However, again, 7-MX did not affect retinal dopamine metabolism.

A possible limitation of our study may be the relatively short treatment time, 13 days in the tube-feeding experiment and 8 days in intravitreal injection experiment. In monkey experiments, treatment was continued for 4 months [14] or 3 weeks in guinea pigs [13]. It is known that extended exposure to the AR antagonist caffeine can

lead to up- or downregulation in the expression of A_1R and $A_{2A}R$ [52], indicating that acute or chronic treatment may lead to different results [53]. Another possible limitation is that we did not establish a dose – response relationship. The selected drug concentration was chosen based on successful suppression in myopia by 7-MX in mammalian species, but differences in receptor composition and abundance across species might exist as well. It is also not known how much 7-MX might have reached the different fundal layers after systemic administration or intravitreal injection. 7-MX was difficult to dissolve, requiring DMSO as a solvent during intravitreal injections. DMSO might interfere with retinal function [54], but since we injected DMSO also as vehicle in the experimental control group and since the measured morphological and biochemical changes in deprived and control eyes fit well with published studies, this option seems less likely.

Summary

We found no effect of 7-MX on the development of deprivation myopia in chickens, no matter whether it was fed or applied by intravitreal injection. 7-MX had also no effect on dopamine and its metabolites in retina, or its release, as measured in the vitreous. Furthermore, 7-MX did not alter the thickness of the fibrous sclera in chicks. Assuming that the target tissue of 7-MX in mammals is the sclera (which is supported by the lack of an effect of 7-MX on retinal dopamine), the differences between chicks and mammals may trace back to their structural differences in the sclera.

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