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Pharmacologically and Edinger–Westphal stimulated accommodation in rhesus monkeys does not rely on changes in anterior chamber pressure☆

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ABSTRACT

This study was undertaken to understand the role of anterior chamber pressure (ACP) during pharmacological and Edinger–Westphal (EW) stimulated accommodation in anesthetized monkeys. Experiments were performed on one iridectomized eye each of 7 anesthetized adolescent rhesus monkeys. Accommodation was induced by EW stimulation ($n = 2$) and intravenous administration of 0.25–4.0 mg/kg pilocarpine ($n = 6$). Accommodative refractive and biometric changes were measured with continuous 60 Hz infrared photorefraction ($n = 6$) and 100 Hz A-scan ultrasound biometry ($n = 1$). An ocular perfusion system was used to measure and manipulate ACP. Pressure was recorded via a 27-gauge needle in the anterior chamber connected to a pressure transducer ($n = 7$). The needle was also connected to a fluid reservoir to allow ACP to be manipulated and clamped ($n = 4$) by raising or lowering the fluid reservoir. In all six pharmacologically stimulated monkeys ACP increased during accommodation, from 0.70 to 2.38 mmHg, four of which showed pressure decreases preceding the pressure increases. Two eyes also showed increases in ACP during EW-stimulated accommodation of 2.8 and 7.2 mmHg. ACP increased with increasing EW stimulus amplitudes ($n = 2$). Clamping or externally manipulating ACP had no effect on resting refraction or on EW and pharmacologically stimulated accommodation in four eyes. The results show that EW stimulated and pharmacologically stimulated accommodation do not rely on ACP in rhesus monkeys.

1. Introduction

Intraocular pressure (IOP) has been suggested to play a role in the accommodative mechanism. The classical Helmholtz theory of accommodation states that ciliary muscle contraction releases zonular tension around the lens equator to allow the lens to become accommodated (Helmholtz von, 1962). Fincham proposed that the accommodative change in shape of the crystalline lens was caused by the elastic capsule molding the lens into an accommodated form (Fincham, 1937). Although the Helmholtz-Fincham capsular theory has been widely accepted, it has been challenged by an alternative IOP pressure based theory in which the zonular-lens diaphragm in each radial section is suggested to act as a catenary. In support of his theory, Coleman (Coleman, 1986) stated: "...differential pressure measurements between the vitreous and aqueous in primate eyes will be presented to document the existence of vitreous support during accommodation." – pg. 853. This theory suggests that a pressure differential between the anterior and vitreous chamber is produced by ciliary muscle contraction and acts on the catenary to cause the lens to become accommodated (Coleman, 1970, 1986; Coleman and Fish, 2001). Coleman (Coleman, 1986) shows pressure recordings from the anterior and vitreous chamber in primate eyes during ciliary muscle stimulation and states: "*In these tracings, an initial rise in vitreous pressure was simultaneously accompanied by a decrease in aqueous pressure. These amplitudes were typically quite small, averaging 4 cm of H_20 and accompanied by a strong piston-like forward movement of the lens similar to that described by Jampel and Mindel (Jampel and Mindel, 1967) with stimulation of the accommodative nucleus of the midbrain of the macaque.*" – *pg. 854*. The initial decrease in anterior chamber pressure (ACP) was suggested to be due to facilitation of aqueous outflow and the increase in vitreous chamber pressure was suggested to be due to ciliary muscle contraction (Coleman, 1986).

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If true, the catenary theory would challenge current understanding of the accommodative mechanism and many current approaches aimed at restoring accommodation to presbyopic eyes which rely on the elasticity of the lens capsule and not on changes in IOP (Glasser, 2006, 2008; Schor, 2009; Sheppard et al., 2010). Knowing if accommodation is affected by IOP would help to understand the accommodative mechanism and the accommodative forces that might be available to allow accommodation restoration strategies to succeed.

In a recent study, enucleated pig eyes were used to develop experimental methods to manipulate IOP (He et al., 2012b). Perfusion-induced pressure increases in the vitreous chamber of pig eyes caused a decrease in lens thickness which is opposite to the accommodative change predicted by the catenary theory. However, the absence of ciliary muscle contraction in enucleated pig eye means that the IOP changes do not necessarily emulate the pressure changes that might occur in a living primate eye with accommodation.

To study the relationship between accommodation and ACP, ideally either accommodation or ACP must be manipulated and the corresponding change in the other variable measured. Rhesus monkeys have an accommodative mechanism similar to humans (Glasser et al., 2006; Glasser and Kaufman, 1999) and a similar relative age-related progression of presbyopia to humans (Bito et al., 1982; Neider et al., 1990). Accommodation can be induced in monkeys by topically or systemically applied muscarinic agonists (Koretz et al., 1987; Ostrin and Glasser, 2007; Wendt and Glasser, 2010) or by Edinger–Westphal (EW) stimulation (Baumeister et al., 2008; Crawford et al., 1989; Vilupuru and Glasser, 2002). Intraocular pressure can be manipulated by changing the height of an ocular perfusion system reservoir (Ethier et al., 1993; He et al., 2012b; Kee et al., 1997). Ocular refractive or biometric changes can be dynamically measured during both accommodation and IOP manipulations (Beers and van der Heijde, 1994; Croft et al., 1998; He et al., 2012b; Ostrin et al., 2006; Vilupuru and Glasser, 2005). This study was undertaken to induce accommodation while manipulating ACP and measuring the ocular responses in rhesus monkeys to understand whether the changes in ACP during accommodation are causal or consequential.

2. Methods

2.1. Animal preparations

All experiments were performed in accordance with an institutionally approved animal protocol and conformed to the ARVO Statement for the Use of Animals of Ophthalmic and Visual Research. Seven adolescent rhesus monkeys, 8–13 years old, were used. Both eyes were previously iridectomized to allow photorefraction to be performed without interference from strong accommodation-induced pupil constriction (Kaufman and Lütjen-Drecoll, 1975; Vilupuru and Glasser, 2002). The iridectomy does not alter the accommodative response (Crawford et al., 1990b) or aqueous humor dynamics (Kaufman, 1979). Two of the monkeys, aged 9 and 13 years, had previously had stimulating electrodes surgically implanted in the EW nucleus of the midbrain (Baumeister et al., 2008; Crawford et al., 1989; Vilupuru and Glasser, 2002). For the experiments, the monkeys were initially anesthetized with intramuscular (i.m.) 15 mg/kg ketamine followed by intravenous (i.v.) propofol (PropoFlo, Abbott Laboratories, North Chicago, IL) with an initial bolus of 1.5 mg/kg and a continuous infusion at 0.5 mg/kg/min. Monkeys were wrapped in a 37 °C water heated pad, intubated and respirated. Pulse rate, SpO_2, and temperature were monitored. In some experiments, anesthesia was supplemented with 0.05 mg/kg i.m. medetomidine (Pfizer Inc., New York City, NY) and/or sutures were placed through medial and lateral rectus muscles to stabilize eye movements. Medetomidine was reversed with 0.25 mg/kg i.m. atipamezole (Pfizer Inc., New York City, NY) at the end of the experiments. For all experiments, the monkeys' head was positioned upright and facing forward in a head holder.

2.2. Sterilized perfusion system and pressure measurement

To allow manipulation and recording of anterior chamber pressure, a perfusion system similar to one described previously was developed and used (He et al., 2012b) (Fig. 1A and B). This consisted of a 5 cm internal diameter, 100 ml fluid reservoir with an outlet tube at the bottom connected to one opening on a 3-way stopcock. A second opening on the stopcock was connected via a plastic tube to a stainless-steel USB pressure transducer with a resolution of 0.00075 mmHg (PR41-X; Keller America Inc., Newport News, VA). The system was sterilized and filled with sterile heparinized Ringer's solution (1 unit/ml; Hospira, Inc., Lake Forest, IL) to prevent blockage. Any air bubbles present were dislodged to the open reservoir. The pressure transducer and the reservoir were each attached with clamps to two 50 cm long vertical posts marked at 2 cm intervals (corresponding to 1.5 mmHg in pressure). The height of the transducer was adjusted on the post to be at the same level as monkey eye.

A sterile 27 G butterfly needle (Becton–Dickinson, Franklin Lakes, NJ) with a short length of fine rubber tubing was attached to the third opening of the 3-way stopcock. Each needle was notched with a micro-file with three rings 1.5 mm from the tip to maintain the needle in the corneal stroma after insertion. Pressure was recorded using a custom Matlab (The Mathworks Inc., Natick, MA) program at 240 Hz. Immediately before the butterfly needle was inserted into the anterior chamber, the stopcock between the transducer and the needle was opened, the pressure transducer was set to the “default zero” factory calibrated zero pressure via the software. As a result, the pressure transducer generally recorded the absolute pressure based on its factory calibration. According to the manufacturer, the Keller pressure transducer has a resolution of 0.00075 mmHg and error range of ±0.075 mmHg. Pressure values, with the system times at which they were recorded, were written to a file once the pressure recording was started. The program also allowed the user to enter events during the experiment which were also recorded to the data file at the corresponding times.

Only one eye of each monkey was used, chosen randomly. The monkey eye lid was held open with a sterile speculum. The eye was viewed with a slit-lamp at high magnifications and the butterfly needle was inserted through clear cornea just anterior to the limbus so the beveled needle tip was completely in the anterior chamber on the temporal side. A custom made sterile, rigid contact lens with a notch 0.5 mm in width and 1.5 mm in length was placed on the cornea to fit around the needle to prevent corneal dehydration (Fig. 1C).

2.3. Pharmacologically and EW stimulated accommodation

Accommodation was stimulated with i.v. pilocarpine in six of the seven monkeys. As described previously (Wendt et al., 2013; Wendt and Glasser, 2010) following an i.m. protective dose of 0.05 mg/kg atropine (Phoenix Pharmaceutical, Inc., St. Louis, MO), several i.v. boluses of pilocarpine (Sigma–Aldrich Corp., St. Louis, MO) varying from 0.25 to 4.0 mg/kg were delivered over 30 s intervals at various times during the experiments using a syringe pump (KD Scientific Inc., Boston, MA). At the end of each i.v. pilocarpine experiment, a 0.5 mg/kg i.v. dose of atropine was given to reverse the effects of the pilocarpine. At the start of each i.v.