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Comparison between carbachol iontophoresis and intravenous pilocarpine stimulated accommodation in anesthetized rhesus monkeys

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ABSTRACT

Rhesus monkeys are an animal model for human accommodation and presbyopia and consistent and repeatable methods are needed to stimulate and measure accommodation in anesthetized rhesus monkeys. Accommodation has typically been pharmacologically stimulated with topical pilocarpine or carbachol iontophoresis. Intravenous (i.v.) pilocarpine has recently been shown to produce more natural, rapid and reproducible accommodative responses compared to topical pilocarpine. Here, i.v. pilocarpine was compared to carbachol iontophoresis stimulated accommodation. Experiments were performed under anaesthesia on five previously iridectomized monkeys aged 10–16 years. In three monkeys, accommodation was stimulated with carbachol iontophoresis in five successive experiments and refraction measured with a Hartinger coincidence refractometer. In separate experiments, accommodation was stimulated using a 5 mg/kg bolus of i.v. pilocarpine given over 30 s followed by a continuous infusion of 20 mg/kg/hr for 5.5 min in three successive experiments with the same monkeys as well as in single experiments with two additional monkeys. Refraction was measured continuously using photorefraction with baseline and accommodated refraction also measured with the Hartinger. In subsequent i.v. pilocarpine experiments with each monkey, accommodative changes in lens equatorial diameter were measured in real-time with video-image analysis. Maximum accommodation of three monkeys with carbachol iontophoresis (five repeats) was (mean ± SD; range) 14.0 ± 3.5; 9.9–20.3 D and with i.v. pilocarpine stimulation (three repeats) was 11.1 ± 1.1; 9.9–13.0 D. The average of the standard deviations of maximum accommodation from each monkey was 0.8 ± 0.3 D from carbachol iontophoresis and 0.3 ± 0.2 from i.v. pilocarpine. The average latency to the start of the response after carbachol iontophoresis was 2.5 ± 3.9; 0.0–12.0 min with a time constant of 12.7 ± 9.5; 2.3–29.2 min. The average latency after i.v. pilocarpine was 0.31 ± 0.03; 0.25–0.34 min with a time constant of 0.19 ± 0.07; 0.11–0.31 s. During i.v. pilocarpine stimulated accommodation in five monkeys, lens diameters decreased by 0.54 ± 0.09; 0.42–0.64 mm with a rate of change of 0.052 ± 0.002; 0.050–0.055 mm/D. Accommodative responses with i.v. pilocarpine were more rapid, consistent and stable than those with carbachol iontophoresis. The accommodative decrease in lens diameter with i.v. pilocarpine as a function of age was consistent with previous results using constant topical pilocarpine. Intravenous pilocarpine stimulated accommodation is safe, more consistent and more rapid than carbachol iontophoresis and it requires no contact with or obstruction of the eye thus allowing continuous and uninterrupted refraction and ocular biometry measurements.

1. Introduction

Rhesus monkeys have been widely used as an appropriate animal model for human accommodation and presbyopia (Bito et al., 1982; Croft et al., 2006). The anatomy of the eye, the accommodative mechanism and the age-course of the progression of presbyopia relative to life span in rhesus monkeys are all similar to humans (Bito et al., 1982; Bito et al., 1987; Glasser and Kaufman, 1999; Lütjen-Drecoll et al., 1988a; Lütjen-Drecoll et al., 1988b). Approaches to restore accommodation have also been investigated in rhesus monkeys (Haefliger and Parel, 1994; Koopmans et al., 2006). Accommodation has been induced in anesthetized

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monkeys in a variety of ways, including with Edinger-Westphal (EW) stimulation, application of muscarinic agonists to the eye or with systemic (intramuscular (i.m.) or intravenous (i.v.)) pilocarpine (Bito et al., 1982; Chin et al., 1968; Crawford et al., 1989; Croft et al., 1998; Haefliger and Parel, 1994; Jampel and Mindel, 1967; Koopmans et al., 2006; Koretz et al., 1987; Neider et al., 1990; Nishi and Nishi, 1998; Tornqvist, 1965, 1966; Vilupuru and Glasser, 2002; Wendt and Glasser, 2010, 2012).

Carbachol iontophoresis or topical pilocarpine are the most common pharmacological methods to stimulate accommodation in anesthetized rhesus monkeys (Haefliger and Parel, 1994; Koopmans et al., 2006; Nishi and Nishi, 1998; Tornqvist, 1964; Vilupuru and Glasser, 2002; Wendt and Glasser, 2010, 2012). Although topical pilocarpine may be the most approachable and straightforward of these, it has been shown to be unreliable (Wendt and Glasser, 2010). Carbachol iontophoresis has been routinely and widely used (Koopmans et al., 2006; Koretz et al., 1987; Vilupuru and Glasser, 2002; Wendt and Glasser, 2012). However, carbachol iontophoresis stimulated accommodative amplitudes are variable in the same monkey (Wendt and Glasser, 2012) and slow (taking 20–30 min to asymptote) (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2002; Wendt and Glasser, 2012). Further carbachol produces an unnatural accommodative response in that although initially the accommodative response is normal with anterior movement of the anterior lens surface and posterior movement of the posterior lens surface, following that the entire lens is translated forward (Ostrin and Glasser, 2005; Vilupuru and Glasser, 2002). It is unclear why the delayed, unnatural forward lens translation occurs, but it may be that high carbachol concentrations delivered into the anterior chamber cause a supra-maximal contraction of the ciliary muscle.

Systemically administered pilocarpine has also been widely used to stimulate accommodation in anesthetized monkeys (Erickson-Lamy et al., 1987; Kaufman and Bárány, 1975; Tornqvist, 1964, 1965, 1967). Tornqvist performed comprehensive studies of i.m pilocarpine stimulated accommodation in monkeys (Tornqvist, 1964, 1965, 1967). However, Tornqvist studies were not on rhesus macaques (*Macaca mulatta*), did not include continuous measurements of accommodation and did not measure accommodative biometric changes that are inherently part of the accommodative response. Tornqvist also identified side effects of systemic pilocarpine which might suggest that it cannot be used safely and effectively. Recently, i.v. pilocarpine stimulation in conjunction with ocular biometry measurements has been shown to produce accommodative responses which are more natural, rapid and reproducible compared to topical pilocarpine (Wendt and Glasser, 2010). The primary objective of this study was to compare the utility, safety, time-course, stability and repeatability of i.v. pilocarpine and carbachol iontophoresis stimulated accommodative refractive changes from repeated experiments in the same monkeys. The secondary objective of this study was to compare the accommodative refractive changes with the accommodative changes in lens diameter during successive i.v. pilocarpine stimulation experiments in five monkeys.

2. Methods

2.1. Animal preparation

All experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were performed in accordance with institutionally approved animal protocols. Experiments were performed on the right, iridectomized, eyes of five rhesus monkeys (*M. mulatta*), between 10 and 17 years of age (monkey numbers 66, 73, 99, 112, 115 with ages in years of 16.6, 11.5, 10.6, 12.6, 13.6 respectively). Throughout the text, the term experiment is referred to as a single procedure or trial in a single experimental session in which one accommodative response was stimulated. Direct comparisons of carbachol and i.v. pilocarpine were made on three monkeys (73, 99, and 112). Monkeys were initially anesthetized with intramuscular 15 mg/kg ketamine and experiments were performed under i.v. propofol (PropoFlo, Abbott Laboratories, North Chicago, IL) anesthesia with an initial bolus of 1.5 mg/kg and a continuous infusion at 0.5 mg/kg/min. For i.v. pilocarpine experiments, monkeys were intubated and respirated, maintained prone on a table with the head held upright and facing forward in a head holder with the eyelids held open with a lid-speculum and a clear plano contact lens on the cornea. Pulse rate, SpO_2, and temperature were monitored. Sutures were tied beneath the lateral and medial rectus muscles to prevent eye movements. Prior to the i.v. pilocarpine stimulation experiments, 0.025 mg/kg i.m. dexmedetomidine (Pfizer, New York, New York) was administered to further reduce eye movements. At the end of the experiments, dexmedetomidine was reversed with 0.25 mg/kg i.m. atipamezole (Pfizer, New York, New York).

2.2. Carbachol iontophoresis stimulated accommodation

In five separate experiments, at least one week apart, carbachol iontophoresis experiments were performed on each of three monkeys to determine repeatability. Carbachol was prepared and delivered iontophoretically for 8 s each on the nasal and temporal sides of the cornea during the initial application and 4 s each in subsequent applications (Koopmans et al., 2006; Koretz et al., 1987; Vilupuru and Glasser, 2002; Wendt and Glasser, 2012). The contact lens was then replaced on the cornea. Static measurements of accommodation were made with a Hartinger coincidence refractometer (Carl Zeiss Meditec, Jena, Germany) from immediately before until up to 100 min following carbachol delivery with three repeated measurements in quick succession at each 2 min interval. Measurements continued until no further increase was observed after three successive 2 min time intervals at which time carbachol iontophoresis was applied a second time. Accommodation measurements continued until a final asymptote was reached.

2.3. Intravenous pilocarpine stimulated accommodation

Three separate i.v. pilocarpine experiments were performed on each of five monkeys, each at least one week apart, to determine repeatability. Accommodation was measured simultaneously using real-time dynamic photorefraction and the Hartinger using a hot mirror beam splitter placed 3 cm in front of the eye at a 45° angle (He et al., 2012; Vilupuru and Glasser, 2002). Photorefraction images were analyzed real-time in a Matlab (The MathWorks, Inc., Natick, MA) program at approximately 15 Hz.

To prevent systemic side effects of the i.v. pilocarpine an initial i.m. dose of 0.015 mg/kg glycopyrrolate was administered (Tornqvist, 1967; Wendt and Glasser, 2010). All subsequent drug administrations and measurements were timed with respect to this initial glycopyrrolate delivery (Fig. 1). Photorefraction measurements started 25 min after glycopyrrolate and five baseline Hartinger measurements were made through the beam splitter at 27 min. At 30 min, pilocarpine was administered via an i.v. catheter in the other leg from the propofol catheter using a syringe infusion pump (KDS210, KD Scientific, Boston) controlled via a Matlab program. An initial i.v. pilocarpine bolus of 5 mg/kg was administered over 30 s immediately followed by a constant infusion of 20 mg/kg/hr for 5.5 min. Another five Hartinger measurements were performed immediately after the pilocarpine constant infusion was completed (at 36 min) when the eye was maximally accommodated.