A DISSERTATION
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Effect of Topical Rocuronium Bromide on Mydriasis and Electroretinography (ERG) in Domestic Pigeons (*Columba livia*)

비둘기(*Columba livia*)에서 **Rocuronium Bromide**의 점안이 산동 및 망막전위도에 미치는 영향

by

Lina Susanti

MAJOR IN VETERINARY CLINICAL SCIENCES
DEPARTMENT OF VETERINARY MEDICINE
GRADUATE SCHOOL
SEOUL NATIONAL UNIVERSITY

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by

Lina Susanti

Supervised by
Professor Kangmoon Seo

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Lina Susanti

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Professor Kangmoon Seo

Lina Susanti

Major in Veterinary Clinical Sciences, Department of Veterinary Medicine,

Graduate School, Seoul National University

ABSTRACT

The purpose of the present study was to investigate the effect of topical application of rocuronium bromide on mydriasis and electroretinography (ERG) in domestic pigeons (*Columba livia*).

In Chapter I, the effect of neuromuscular blocking agent rocuronium bromide on the mydriasis of pigeons was investigated. This investigation was conducted in two phases. In the first phase, a single dose of 0.20 mg/20 μL rocuronium bromide was instilled topically to the right eye (OD) of eight domestic pigeons. The iris

colors of these pigeons consisted of gravel, pearl, and bull eye. Pupil diameter and pupillary light reflex (PLR) were measured before instillation at the time base (T0), and at 5 (T05), 10 (T10) minutes after instillation, and every 10 minutes thereafter until 160 (T160) minutes. The pupil diameter was measured in millimeter (mm) and the PLR was assessed using a scoring system. Rocuronium bromide produced mydriasis in 6/8 (75%) pigeons in the first phase of the investigation. On those six pigeons, the maximal mydriasis was observed at T30 with a mean pupil diameter of 4.62 ± 0.13 mm. Pupil diameter measurement in the treated eye was significantly different from that in the contralateral eye and from T0 since T05 – T120 ($P < 0.05$). The PLR was disappeared from T10 to T90 ($P < 0.05$). In the second phase of the investigation, the same dosage was instilled twice in the span of 10 minutes into both eyes (OU) of four pigeons (eight eyes). In this phase, all the pigeons had a bull eye iris. The same measurement of pupil diameter and PLR were performed at the same time points. Mydriasis was only observed in 2/8 eyes. This suggested that topical rocuronium bromide was able to produce mydriasis on pigeons other than bull eye iris. During the study, no significant side effects were observed except for a transient lower eyelid paresis.

In Chapter II, the effect of mydriasis with topical rocuronium bromide on the ERG of domestic pigeons was investigated. In this study, scotopic mixed rod and cone, photopic cone, and photopic flicker ERG were performed on nine adult healthy pigeons under sedation with and without the induction of mydriasis using the topical application of rocuronium bromide. No statistically significant differences were observed during the scotopic mixed rod and cone ERG between non-mydriatic and mydriatic conditions. During the photopic ERG examinations,

however, significant differences were observed on both the photopic cone and photopic flicker amplitudes of ERG between non-mydriatic and mydriatic conditions. The amplitudes of the photopic ERG were significantly higher in the mydriatic condition.

Based on the results of the current study, topical application of rocuronium bromide reliably produced mydriasis on pigeons with iris colors other than bull eyes without significant side effects. The degree of mydriasis achieved by the topical rocuronium bromide manage to facilitate advanced ophthalmic examination such as ERG in pigeon. In pigeon, the amplitude of photopic ERG in mydriatic eye induced with rocuronium bromide was significantly higher. In conclusion, rocuronium bromide can be safely used as a mydriatic agent in pigeons to facilitate general ophthalmic examination and ERG examination.

Keywords: *Columba livia*, domestic pigeon, electroretinography, mydriasis, rocuronium bromide.

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List of Abbreviations

ANOVA	analysis of variance
GABA	gamma-aminobutyric acid
cd m^{-2}	candela per square meter (luminous intensity)
cd s m^{-2}	candela times second per square meter (time integrated luminance)
cm	centimeter
ERG	electroretinography
Hz	hertz
kg	kilogram
mg	milligram
mm	millimeter
ms	millisecond
OP	oscillatory potential
µg	microgram
µV	microvolt

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CHAPTER II

Effect of Mydriasis with Topical Rocuronium Bromide on Electroretinography (ERG) in Domestic Pigeons (*Columba livia*)

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GENERAL INTRODUCTION

In avian patients, owing to the difference in ocular anatomy and physiology, performing ophthalmic examinations can be challenging when compared with other veterinary patients such as dogs and cats (Holmberg, 2018). Among these anatomical differences was the fact that in many avian species, the iris musculature consisted mainly of striated muscle as opposed to iris in mammals (Oliphant *et al*., 1983; Barsotti *et al*., 2010b; Holmberg, 2018). As a result, inducing mydriasis in avian patients is often difficult because parasympatholytic and sympathomimetic agents commonly used to induce mydriasis in mammals' irises do not work in avian patients (Kern and Colitz, 2013).

Inducing mydriasis during ophthalmic examination is often crucial especially during the examination of the posterior segment of the eye, such as during electroretinography (ERG) test (Maggs, 2018a). ERG records the electrical activity of the retina and is commonly used in veterinary ophthalmology to evaluate retinal function and diagnose retinal diseases (Ekesten, 2013). During ERG test, mydriasis is essential because performing ERG test in a miotic pupil can result in reduced amplitude and/or prolonged implicit time caused by limited light that reached the retina (Ekesten, 2013). However, owing to the difficulty of inducing mydriatics in birds, many reports of ERG performed in avian species had been done without the induction of mydriasis (Hendrix and Sims, 2004; Labelle *et al*., 2012; Seruca *et al*., 2012; Kuhn *et al*., 2014; Susanti *et al*., 2019).

In pigeons, several compounds have been investigated on their ability to induce mydriasis, including intracameral injection of *d*-tubocurarine which possessed certain complications such as intraocular infections and cataract formation (Verschueren and Lumeij, 1991). Aside from that, topical applications of curare and gallamine had also been investigated with inconsistent results (Campell and Smith, 1962). Therefore, in Chapter I, the effect of topical rocuronium bromide in inducing mydriasis in pigeons was evaluated. Rocuronium bromide, a neuromuscular blocking agent, had been reported to be a safe mydriatics when applied topically in many species of birds (Barsotti *et al*., 2010a; 2010b; 2012; Baine *et al*., 2016; Petritz *et al*., 2016). In the Chapter II, ERG test performed in pigeons without the induction of mydriasis were compared with ERG test with the induction of mydriasis using topical application of rocuronium bromide.

CHAPTER I

Efficacy of Topical Rocuronium Bromide as Mydriatic Agent of Domestic Pigeons (*Columba livia*)

Abstract

This study was conducted to investigate the efficacy of rocuronium bromide as mydriatic agent in domestic pigeons (*Columba livia*). This study was done in two phases. In the first phase, rocuronium bromide (0.20 mg/20 μL) was topically instilled to the right eye (OD) of eight domestic pigeons. Pupil diameter was measured before instillation (T0), and at 5 (T05) and 10 (T10) minutes after instillation, and every 10 minutes thereafter until 160 (T160) minutes. Pupillary light reflex (PLR) was assessed using a scoring system at the same time points. In the second phase, the same dosage was instilled twice in the span of 10 minutes into both eyes (OU) of four pigeons (eight eyes). Measurements were done accordingly. The iris color in the first phase were: gravel, pearl and bull eye. All irises in the second phase were bull eye. Mydriasis was observed in 6/8 (75%) pigeons in the first phase. Maximal mydriasis was observed at T30 (mean pupil diameter = 4.62 ± 0.13 mm). Pupil diameter in the treated eye was significantly different from contralateral eye and from T0 since T05 (P = 0.006 and P = 0.017, respectively) – T120 (P = 0.044 and P = 0.043, respectively). PLR was disappeared from T10 (P = 0.041) to T90 (P = 0.034). In the second phase, mydriasis was only observed in 2/8 eyes. This study suggested that rocuronium bromide was able to produce mydriasis in pigeons other than bull eye iris.

Introduction

Mydriatic agents are important diagnostic tools in ophthalmic examinations, especially for evaluating the posterior segment of the eye (Maggs, 2018a). In exotic animals, including birds, the small globe increases the difficulty of critical examination of the lens and posterior segment such as vitreous body, retina, and pecten. One of the examples of such critical examination was after the incident of bird collision, in which, the clinician will need to examine the inside of the eyes. In such cases, pharmacologic mydriasis may facilitate the entire eye examination in birds (Holmberg, 2018).

In avian patients, it could be troublesome to induce mydriasis since the irises of the birds have a different muscular system than those of the mammals, which possess both striated muscle fibers and nonstriated fibers (Oliphant *et al.*, 1983; Barsotti *et al.*, 2010b; Holmberg, 2018). Therefore, parasympatholytic and sympathomimetic agents that are commonly used to induce mydriasis in mammals' irises do not work in avian patients as the latter have predominantly striated fibers in nature (Kern and Colitz, 2013).

In pigeons, several compounds have been investigated to induce mydriasis including *d*-tubocurarine (Verschueren and Lumeij, 1991), curare, and gallamine (Campell and Smith, 1962). The use of *d*-tubocurarine was considered to be impractical as it needs to be administered via intracameral injection which could predispose the eyes to several complications such as intraocular infections and cataract formation (Verschueren and Lumeij, 1991). Conversely, topical applications of curare and gallamine are easy to apply but they give fairly inconsistent results even though benzalkonium chloride is added to help reduce the surface tension of the cornea (Campell and Smith, 1962).

Recently, rocuronium bromide has been reported as an efficient mydriatic agent in several species of birds, including Hispaniolan Amazon parrots (*Amazona ventralis*) (Baine *et al*., 2016; Petritz *et al*., 2016), common buzzard (*Buteo buteo*), little owl (*Athene noctua*), tawny owls (*Strix aluco*) (Barsotti *et al*., 2010a; 2010b), and European kestrels (*Falco tunninculus*) (Barsotti *et al*., 2012). Topical application of rocuronium bromide was reported to be relatively safe, with the majority of bird species experiencing no major side effects, except one report of corneal ulceration (Baine *et al*., 2016) and a transient lower eyelid paresis in Hispaniolan Amazon parrots (Petritz *et al*., 2016). Despite such abundancy of reports in other species, rocuronium bromide has not been investigated in pigeons. This study aimed to investigate the efficacy and safety of rocuronium bromide as a mydriatic agent in pigeons.

Materials and Methods

1. Experimental animal

Twelve healthy adult domestic pigeons (*Columba livia*) of undetermined sex, obtained from a pigeon farm, were used in this study. This study was approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-181224-5). The eyes and periocular region were examined for gross abnormalities in a well-lit room. Intraocular pressure (IOP) measurements (TonoVet®; Icare; Tiolat, Helsinki, Finland; rebound tonometer) were obtained and the adnexa and anterior segment were examined with slit-lamp biomicroscopy (Keeler® PSL One Portable Slit Lamp; Keeler Ltd, Windsor, UK).

2. Experimental procedures

This study was done in two phases. In the first phase of the study, rocuronium bromide (0.20 mg/20 μL) was instilled once into the right eye (OD) of eight birds. The pupil diameter prior to the administration of rocuronium bromide was measured at the time base (T0). The birds were manually restrained and held in lateral recumbency during the instillation of rocuronium bromide with the cornea positioned upward and were maintained in that position for at least 15 seconds to facilitate drug absorption. After the instillation of rocuronium bromide, the pupil diameter was measured at 5 (T05) and 10 (T10) minutes time points and every 10 minutes thereafter, until the pupillary light reflex (PLR) briskly returned at 160 (T160) minutes. The measurement was performed by placing a ruler in contact with the dorsal surface of the eye at a distance of 2 mm from the upper eyelid and its photograph was obtained using a cellular iPhone® SE camera without the use of flash under the same room light condition. The measurement was also performed at the contralateral eye (OS) at every time point.

Direct PLR was evaluated using a finnoff (WA 41100®; Welch Allyn, Skaneateles Falls, NY, USA) in a darkened room at every time point after the measurement of pupil diameter. The direct PLR was evaluated using a scoring system (0: normal, 1: decreased, 2: almost disappeared, 3: absent).

To further examine the ability of rocuronium bromide to induce mydriasis in pigeon with bull eye colored iris, the second phase of the study was conducted. In the second phase of the study, rocuronium bromide (0.20

mg/20 μL) was instilled into both eyes (OU) of four birds (eight eyes). The PLR and diameter of the pupil were measured the same way as phase one of the study. In this phase of the study, when rocuronium bromide were failed to induce the mydriasis after 10 minutes, the second dosage were instilled and the measurement were done accordingly.

Throughout the course of the experiment, the animals were monitored for any sign of side effects, including ocular irritation, lacrimation, blepharospasm, conjunctival hyperemia, chemosis, and paralysis of the eyelid, wing, hind limb, or neck.

3. Statistical Analyses

Data were presented as the mean ± standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). A paired student's *t*-test was used to compare pupillary diameter at all time points to T0 and the contralateral eye. The Wilcoxon signed-rank test was used to evaluate the PLR at every time point compared with T0. Differences were considered statistically significant at $P \leq 0.05$.

Results

Result of general ophthalmic examination of the experimental pigeons was normal. IOP ranged from 10 to 15 mmHg. Rocuronium bromide successfully induced mydriasis in six out of eight pigeons used in the first phase of this study. In order to investigate the degree of mydriatic effect induced by rocuronium bromide in pigeons, only the data from the six pigeons in which the drug successfully induced mydriasis were included in the statistical analysis. The effect was immediately observed 5 minutes after the application of rocuronium bromide in pigeons in which mydriasis was induced. Optimal mydriasis was observed at T30 with a mean diameter of 4.62 ± 0.13 mm (Fig. 1). Pupillary diameter of OD was significantly different from that at T0, starting from T05 ($P = 0.006$) up to T120 ($P = 0.044$; Fig. 1; Table 1). Pupillary diameter of OD also showed significant differences compared with the OS starting from T05 ($P = 0.017$) up to T120 ($P = 0.043$; Fig. 1; Table 1). The PLR was disappeared from T10 ($P = 0.041$) to T90 ($P = 0.034$; Table 2).

In the first phase of the study, three different colors of iris were observed among the eight birds: gravel (1/8), pearl (3/8), and bull eye (4/8). The two eyes in which rocuronium bromide were failed to induce mydriasis were both had bull eye color. In the second phase of the study, all of the birds had bull eye colored irises (8 eyes out of four birds). In this phase of the study, the first instillation of rocuronium bromide was failed to induced mydriasis in all of the eyes. The second instillation of rocuronium bromide were managed to induce mydriasis in two of the eight eyes. Because of the lack of efficacy, statistical analysis was not performed for the second phase of the study.

During the topical instillation of the drug, all pigeons in this study experienced irritation of variable degrees with three pigeons reacting more strongly as compared with the others. All pigeons remained alert and responsive during the experiment. Five pigeons in the first phase of the study had transient lower eyelid paresis, which resulted in transient lower eyelid elevation during which the ability of blinking was retained. This was not observed during the second phase of the study. Besides these, no other local or systemic adverse effects were observed.

Table 1. Comparison of mean pupil diameter of domestic pigeons OD after application of rocuronium bromide

Time (minute)	Mean pupil diameter, mm[a)]		*P value*[b)]	
	OD	OS	Compared to T0	Compared to OS
0	3.04 ± 0.18	3.16 ± 0.30		0.296
5	3.91 ± 0.37	2.95 ± 0.18	0.006*	0.017*
10	4.25 ± 0.38	2.95 ± 0.29	0.001*	0.003*
20	4.58 ± 0.12	2.95 ± 0.18	0.000*	0.000*
30	4.62 ± 0.13	2.95 ± 0.29	0.000*	0.000*
40	4.50 ± 0.27	2.91 ± 0.12	0.000*	0.000*
50	4.50 ± 0.38	3.08 ± 0.20	0.001*	0.001*
60	4.45 ± 0.40	3.12 ± 0.20	0.001*	0.001*
70	4.37 ± 0.44	2.95 ± 0.29	0.001*	0.002*
80	4.33 ± 0.43	3.00 ± 0.27	0.002*	0.005*
90	4.20 ± 0.55	2.91 ± 0.20	0.005*	0.007*
100	4.12 ± 0.86	2.87 ± 0.13	0.032*	0.019*
110	4.08 ± 0.75	2.91 ± 0.12	0.022*	0.016*
120	3.91 ± 0.75	2.91 ± 0.20	0.044*	0.043*
130	3.87 ± 0.80	3.04 ± 0.18	0.061	0.070
140	3.70 ± 0.74	2.95 ± 0.10	0.112	0.080
150	3.79 ± 0.90	3.00 ± 0.15	0.118	0.112
160	3.58 ± 0.73	2.91 ± 0.12	0.143	0.116

a) Mean ± SD.b) *$P < 0.05$, statistically significant.

Table 2. Comparison of mean PLR scores of domestic pigeons in the right eye (OD) after application of rocuronium bromide from T0 minute

Time (minute)	PLR[a]	*P value*
0	0.00 ± 0.00	
5	2.20 ± 1.30	0.059
10	2.80 ± 0.44	0.034*
20	3.00 ± 0.00	0.025*
30	3.00 ± 0.00	0.025*
40	3.00 ± 0.00	0.025*
50	3.00 ± 0.00	0.025*
60	3.20 ± 1.09	0.039*
70	2.40 ± 0.54	0.038*
80	2.00 ± 1.00	0.041*
90	2.00 ± 1.00	0.041*
100	1.60 ± 1.34	0.063
110	1.60 ± 1.34	0.063
120	1.20 ± 1.30	0.109
130	0.60 ± 0.89	0.180
140	0.60 ± 0.89	0.180
150	0.40 ± 0.89	0.317
160	0.20 ± 0.44	0.317

[a] Mean ± SD. PLR: Pupillary light reflex (0: normal, 1: decreased, 2: almost disappeared, 3: absent).

*$P < 0.05$, statistically significant.

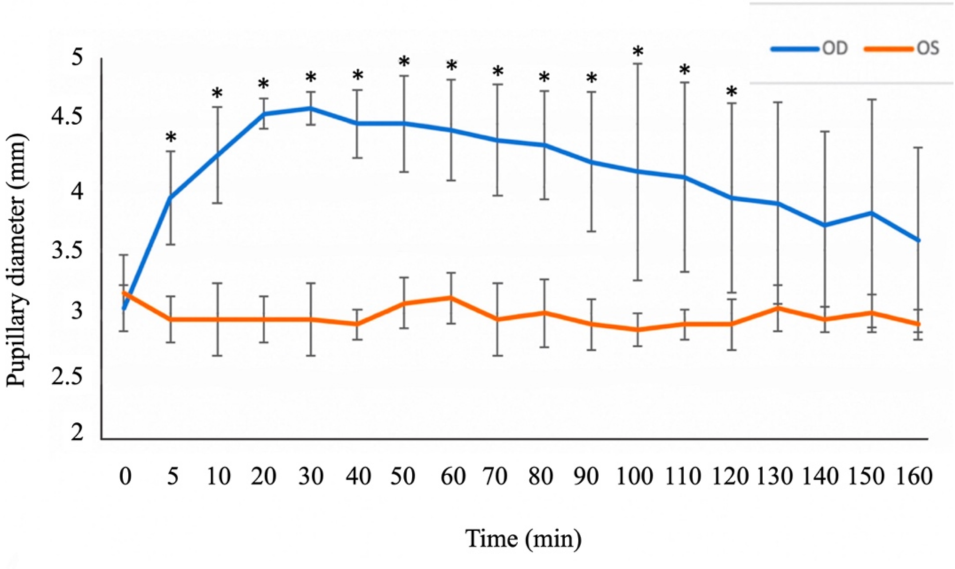

Figure 1. The mean pupillary diameter of the treated eye (OD) and the contralateral eye (OS).

*Significantly different from the T0 and contralateral eye ($P < 0.05$).

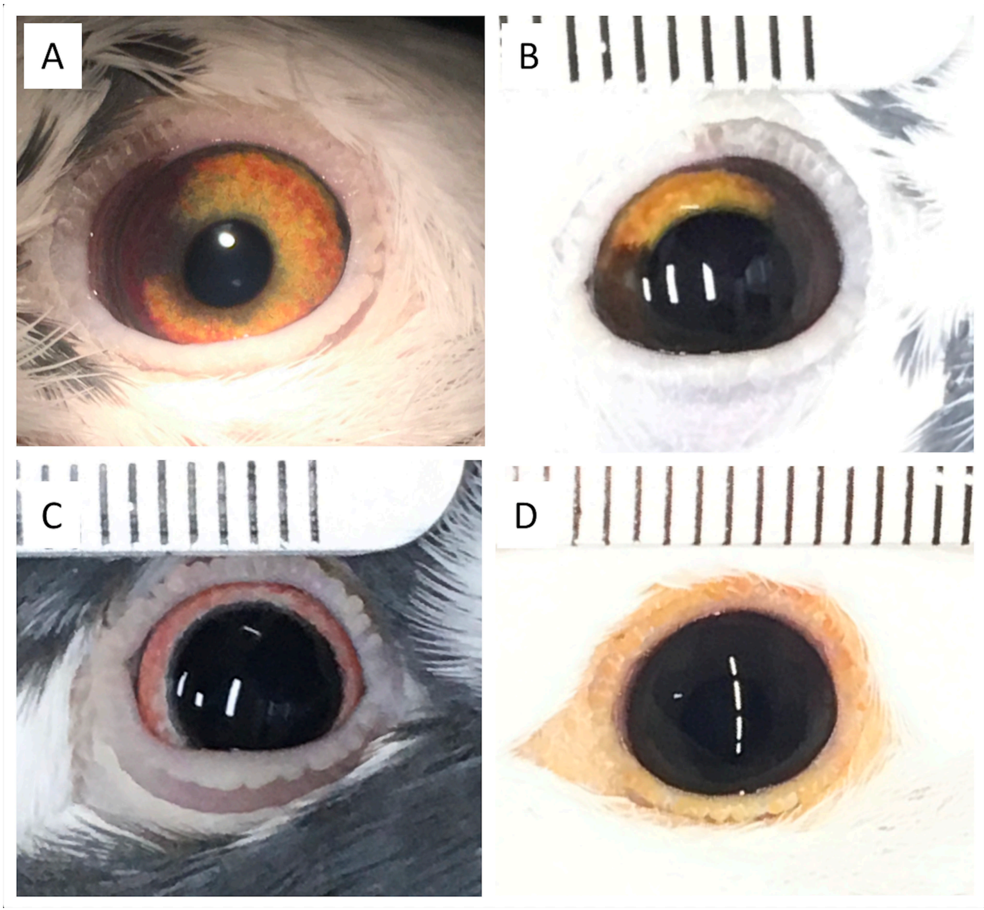

Figure 2. The iris colors in pigeons. (A) Gravel colored iris (partial) during miosis, (B) Partially gravel colored iris during mydriasis, (C) Pearl colored iris during mydriasis, (D) Bull eye iris during miosis.

Discussion

Rocuronium bromide has been widely reported to be a safe and efficient topical mydriatic agent to induce mydriasis in several species of birds, including Hispaniolan Amazon parrots (Baine *et al*., 2016; Petritz *et al*., 2016), common buzzard, little owl, tawny owl (Barsotti *et al*., 2010a; 2010b), and European kestrels (Petritz *et al*., 2016). In previous reports, rocuronium bromide managed to produce consistent mydriasis in 100% of the birds (Barsotti *et al*., 2010a; 2010b; 2012; Baine *et al*., 2016; Petritz *et al*., 2016). In the first phase of the present study, however, rocuronium bromide was only effective in 6/8 (75%) of the pigeons. With striated muscle fibers known to be predominant in the birds' irises, neuromuscular blocking agents are expected to produce mydriasis in birds (Kern and Colitz, 2013; Samuelson, 2013). However, there seem to be variations in the arrangement and development of the various muscular components among species of birds (Oliphant *et al*., 1983; Peirone *et al*., 1990; Samuelson, 2013; Baine *et al*., 2016), which is probably why the efficacy of rocuronium bromide in pigeons in this study was different from that reported previously (Barsotti *et al*., 2010a; 2010b; 2012; Baine *et al*., 2016; Petritz *et al*., 2016).

Another possible explanation for the lack of efficiency of rocuronium bromide in inducing mydriasis in this study might be related to the different iris coloration among the pigeons used. Three iris types are known to exist in pigeons: the wild type or "gravel," the white or "pearl," and the "bull eye," which is almost black (Staples-Browne, 1908); all three variations existed in pigeons in the first phase of the study. The two pigeons that did not respond to rocuronium bromide had a greyish brown-colored iris or what is known as "bull eye." In the second phase of the study, all of the eyes also had bull eye irises. These different responses from different iris colors to rocuronium bromide are similar to the observation from its parent compound, vecuronium bromide, when used as a mydriatic agent in double-crested cormorant (*Phalacrocorax auritus*) in which vecuronium bromide was effective in inducing mydriasis in an adult cormorant with a blue-colored iris but was only marginally effective and highly inconsistent when used in juvenile cormorants with a brown-colored iris (Loerzel *et al*., 2002).

Interestingly, not all pigeons with greyish brown-colored iris or "bull eye" in this study failed to respond to single instillation of rocuronium. It is important to note that even though only eight pigeons were included in the first phase of the study, during the preliminary study, a single instillation of rocuronium bromide was effectively induced mydriasis in all pigeons with such iris color. Another possible reason for this failure could be that the volume of the drugs instilled could not be maximally absorbed. Although it was ensured that all dosages were retained at the eye fissure during the instillation it was observed that several pigeons made the swallowing gesture several times, which might have resulted in the drug being rapidly drained through the nasolacrimal duct before it was absorbed in the eyes. However, observing the result form the second phase of the study, in which rocuronium were given twice, it was considered that the different iris coloration might be the reason for these different results.

In the cormorant study, it was suspected that the different pigmentation might have resulted in pigment-binding effect of the vecuronium in the brown-colored iris, affecting the availability of the drug as it bound to the melanin and therefore, failed to induce effective mydriasis (Loerzel *et al*., 2002). The tendency of the drug compound to bind to the melanin has been shown in several experiments and is known to influence ocular pharmacokinetics of the drugs that it is usually associated with prolonged drug retention in the pigmented tissue leading to prolonged drug responses (Samuelson, 2013; Rimpela *et al*., 2018). In pigeons, different iris coloration is associated with the presence or absence of the pigments, guanine and pteridine, in the anterior surface of the iris (Oliphant, 1987a; 1987b). In pigeons, the color gravel (yellowish red; Fig. 2A, B) is associated with the presence of both guanine and pteridine pigments while the color pearl (white with varying tinges of red to pink variations due to abundant blood vessels) (Fig. 2C) is associated with the guanine pigment (Oliphant, 1987a; 1987b). The absence of both the pigments in the anterior surface of the iris results in the "bull eye," (Fig. 2D) in which the posterior hexagonal brown or black pigment shows through and thus produces the black color effect (Bond, 1920).

Considering that the same iris color in different species of birds could be caused by different mechanisms (Bond, 1920; Samuelson, 2013), whether it is the existence of certain pigment or the lack of it (Bond, 1920), it is difficult to conclude whether the failure of rocuronium to induce mydriasis in a pigeon with "bull eye" iris is caused due to the same reason as vecuronium's lack of effect in juvenile cormorant, without the knowledge of the nature of cormorant iris color pigmentation. In fact, the majority of black or very dark brown iris in birds owe their dark color to the presence of anterior iris pigment and are different from the "bull eye" irises in pigeons (Bond, 1920). That being said, it will be interesting to investigate the effect of rocuronium on the same species of birds with a different iris color to understand whether rocuronium has the tendency to bind to certain iris pigment and whether or not it is affecting the pharmacokinetics of the rocuronium. In the present study, the pigeons were obtained randomly from a pigeon farm; therefore, the variation in color of the irises in these pigeons could not be controlled. Furthermore, because of the small number of pigeons used in this study, there is also a possibility for rocuronium to fail to induce mydriasis in pigeons with gravel and pearl eyes even though, in this experiment, mydriasis was induced successfully in irises of those colors.

The dosage of rocuronium used in this study was established based on a previously published study on other species (Barsotti *et al*., 2010a; 2010b; 2012; Baine *et al*., 2016; Petritz *et al*., 2016). Smaller dosage was recommended with regard to safety precautions over the possible side effects since neuromuscular blocking agents are known to have caused systemic adverse effects such as eyelid, neck, and hind-limb muscle paralysis when used as mydriatic agents in birds (Mikaelian *et al*., 1994; Barsotti *et al*., 2012). Previous studies on rocuronium bromide on other species did confirm the safety profile of rocuronium bromide as a mydriatic (Barsotti *et al*., 2010a; 2010b; 2012; Baine *et al*., 2016; Petritz *et al*., 2016); the only side effects reported were superficial corneal ulceration, either caused by restrain or by corneal irritation from the acidity of the drugs (Baine *et al*., 2016) and a transient lower eyelid paresis (Petritz *et al*., 2016). Rocuronium bromide is known to have a low pH (Baine *et al*., 2016) and therefore, might cause irritation when applied topically to the eyes. During the instillation of the drugs in this study, all pigeons seemed to have experienced a variable degree of irritation, with three pigeons reacting more strongly as compared with the others.

In the first phase of the study, five pigeons experienced transient lower eyelid paresis, in which the lower eyelid was partially elevated. During this transient lower eyelid paresis, the ability to blink was not affected, which was similar to the observation in Hispaniolan Amazon parrots (Petritz *et al*., 2016). None of the birds in the second phase of the study experienced this complication. Lower eyelid paresis was also reported in a study of another neuromuscular blocking agent, alcuronium in Hispaniolan Amazon parrots in which full neck and hind-limb muscle paralysis was also observed (Mikaelian *et al*., 1994). During the course of the present study, transient lower eyelid paresis without any other side effects, except irritation, were observed.

Hodos *et al*., (1998) used vecuronium in their study to produce mydriasis in pigeons through a series of drops every 60 seconds during the course of 6–10 minutes and managed to induce mydriasis of 5.75–6.0 mm in diameter. In the present study, the maximum mydriasis in pigeons induced by a single topical application of rocuronium bromide resulted in a median of 4.62 ± 0.13 mm in diameter with one pigeon achieving a maximum of 5.25 mm of mydriasis. Compared to vecuronium, rocuronium is known to only have one-eighth of the vecuronium potency (Keegan, 2015), which might explain the superior mydriasis achieved in the former study. Conversely, although it is impossible to exactly know the dosage of vecuronium in the previous study (Hodos *et al*., 1998), when it was considered that one drop is equal to 0.05 mL, each pigeon in the previous research received approximately 3–5 mg of vecuronium compared with only 0.20 mg rocuronium in the present study, which is probably the reason for the wider diameter of the pupil mydriasis in the former study.

Although rocuronium lacks potency compared to its parent compound, rocuronium is generally known to have a more rapid onset of action (Keegan, 2015). In theory, having a lower potency means that rocuronium is safer to be applied in a larger dosage than vecuronium, and since both drugs have similar molecular weights, the application of a higher dosage of rocuronium will introduce more molecules into the neuromuscular junctions, increasing the availability of rocuronium to bind to the ACh receptors, resulting in a more rapid onset of neuromuscular blockade (Hunter, 1996). The problem with that design is the small size of pigeon eyes. The rocuronium used in this study had a concentration of 10 mg/mL, which was the same concentration used in another study (Barsotti *et al*., 2010a; Petritz *et al*., 2016) and 0.20 mg was contained in 20 μL. The maximum amount of fluid reported to be able to be held in the human palpebral fissure was only 25–30 μL (Mishima *et al*., 1966; Regnier, 2013). Taking that into consideration, even the dosage used in the current study was unlikely to be retained and absorbed completely by the pigeon (Regnier, 2013). That being said, it was interesting to note that the average time needed for rocuronium to produce 4.00–4.75 mm mydriasis in the present study was 10 minutes after the drug application compared with the previous study in which mydriasis was achieved at 10 minutes after the last drop, which meant 20 minutes after the 1st drug application. A study comparing a single and two consecutive instillations of rocuronium bromide in tawny owls showed no significant differences in mydriasis onset, time for maximal effect, or maximal pupillary diameter (Barsotti *et al*., 2010a). However, given that in the present study, a single instillation of rocuronium bromide failed to induce 100% mydriasis in all pigeons, further studies to investigate repeated instillation of rocuronium bromide in pigeons might be beneficial.

In the first phase of this study, rocuronium bromide only successfully induced mydriasis in 6/8 of the pigeon (75%) and only the data from those six pigeons were used in the statistical analysis in order to investigate the degree of mydriasis produced by rocuronium bromide in pigeons. By doing so, it can be concluded that although rocuronium did not always successfully induce mydriasis in pigeons, when it successfully induced mydriasis, the degree of the dilated pupil was significantly different from the normal pupil.

The duration of mydriasis induced by rocuronium bromide in this study was relatively short compared to previous studies on other birds, which was only less than 3 hr compared to 6 hr in Hispaniolan Amazon parrots (Petritz *et al*., 2016). The short mydriatic duration would be favorable as it could minimize unnecessary light exposure from a prolonged mydriasis. Even though it was reported that there was no evidence of animals undergoing mydriasis (using tropicamide or atropine) to suffer retinal damage from the ambient light level (Maggs, 2018b), it has been demonstrated in laboratory animals (Williams *et al*., 1985).

Conclusions

In conclusion, rocuronium bromide produced moderate mydriasis in pigeons in this study without significant side effects. In the first phase of study population with all iris colors, a single instillation of rocuronium bromide was effective in 6/8 (75%) of pigeons. In the second phase of the study population with only bull eye iris color, a single instillation of rocuronium bromide was not effective in inducing the mydriasis with only 2/8 eyes being induced after twice instillations. While the mydriasis produced by rocuronium bromide was only moderately effective in the first phase of the study, the degree of mydriasis achieved allowed for fundus examinations with direct ophthalmoscopy.

CHAPTER II

Effect of Mydriasis with Topical Rocuronium Bromide on Electroretinography (ERG) in Domestic Pigeons (*Columba livia*)

Abstract

This study aimed to investigate the effect of mydriasis using topical rocuronium bromide on ERG in domestic pigeons (*Columba livia*). Scotopic mixed rod and cone, photopic cone, and photopic flicker ERG were performed on nine eyes of nine healthy adult pigeons under sedation. Each pigeon underwent two sets of ERG recordings: First, without the induction of mydriasis (control) and the second time with the induction of mydriasis using topical rocuronium bromide (treatment). The results were compared using either the Student's *t*-test or Wilcoxon rank-sum test, where a *P*-value of < 0.05 was considered statistically significant. No significant differences were observed in the a- and b-wave implicit times and amplitudes during scotopic ERG between the two groups. The a- and b-wave amplitudes in the photopic cone were significantly higher in the treatment group (63.83 ± 32.33 and 191.75 ± 94.46 µV) compared to the control group (46.15 ± 27.60 and 116.76 ± 70.65 µV; *P* = 0.045 and *P* = 0.032, respectively). The photopic flicker amplitude was also significantly higher in the treatment group (76.23 ± 48.56 µV) than in the control group (42.18 ± 31.18 µV; *P* = 0.044). No statistically significant differences were observed in the photopic cone and flicker implicit times between both groups. In conclusions, mydriasis induced by rocuronium bromide in pigeon resulting in higher amplitudes during the photopic ERG but not scotopic ERG.

Introduction

Electroretinography (ERG) measures the electrical potential generated by the retina in response to changes in illumination (Frishman, 2018). It is the most widely used electrodiagnostic test in veterinary ophthalmology and is commonly used to diagnose outer retinal disease (Ekesten, 2013). ERG is considered a useful diagnostic tool to investigate retinal function as it can be performed non-invasively (Ekesten, 2013). However, in order to obtain a reliable ERG recording, several technical aspects must be addressed, including an equal light stimulation of photoreceptors in all parts of the retina (Ekesten, 2013; Ekesten *et al*., 2013).

During the ERG recording, a mydriatic pupil is essential because a miotic pupil can reduce the amount of light that reaches the retina, which will reduce the amplitude and prolong the implicit time obtained during the ERG (Ekesten, 2013). However, inducing mydriasis in birds is considered challenging because the birds' iris has a different muscular system, and the mydriatic agent commonly used in mammals does not work (Kern and Colitz, 2013). Because of that, several reports on electroretinography on birds had been performed without the induction of mydriasis (Hendrix and Sims, 2004; Labelle *et al*., 2012; Seruca *et al*., 2012; Kuhn *et al*., 2014; Susanti *et al*., 2019). Recently, rocuronium bromide had been reported to be a reliable and safe mydriatic agent in several species of birds (Barsotti *et al*., 2010a; 2010b; 2012; Baine *et al*., 2016; Petritz *et al*., 2016; Dongo *et al*., 2017) including pigeon (Susanti *et al*., 2021). This study was conducted to investigate the difference between ERG recordings with and without pupil dilation in a small-eyed bird such as pigeons.

Materials and Methods

1. Experimental animals

Nine healthy adult domestic pigeons (*Columba livia*) without retinal abnormalities of undetermined sex were used in this study. The eyes and periocular region were examined for gross abnormalities in a well-lit room. IOP measurements were performed using a rebound tonometer (TonoVet; Icare; Tiolat, Helsinki, Finland), and examinations of the adnexa and anterior segment were performed with a slit-lamp biomicroscopy (Keeler PSL One Portable Slit Lamp; Keeler Ltd, Windsor, UK). Fundus imaging was taken using a hand-held retinal camera (GENESIS; Kowa, Tokyo, Japan). This study was approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-190922-1).

2. Electroretinography

ERG recordings were performed on different day from the general eye examinations to avoid the excessive light exposure to the retina that might interfere with ERG recordings. Each pigeon underwent two sets of ERG recordings on the right eye only. The first set of ERG recordings was performed without mydriasis induction, which acted as the control group. After the first ERG set was performed, mydriasis was induced before the second dark adaptation stage for the second set of ERG recordings or the treatment groups. Mydriasis was induced using a single topical instillation of 0.20 mg/20 μL rocuronium bromide (Rocunium Inj. 10 mg/mL, Hana Pharm. Co., Ltd., Kyonggi-do, Korea) on the ocular surface. Because the bone separating the right and the left eyes of the pigeon is so thin, a light shone in one eye can be seen on the contralateral eyes. Therefore, in order to avoid the possibility of the first ERG test (of the first eye) interfering with the second test (of the second eye), only the right eye was used in this study.

The ERG recordings were performed under sedation using 10 mg/kg of alfaxalone (Alfaxan 10 mg/mL, Jurox, Rutherford, Australia) injected intramuscularly into the pectoral muscle before the dark adaptation stage. This dosage was only sufficient to induce sedation in pigeon. Three ERG recordings: 1) scotopic (dim-light) mixed rod and cone response, 2) photopic (bright light) cone response, and 3) photopic flicker response were performed using a RETIport (Roland Consult Stasche & Finger GmbH, Brandenburg, Germany). A 0.25 mm gold-ring electrode (3325RC; Roland

Consult Stasche & Finger GmbH, Brandenburg, Germany) was used as the corneal electrode, and platinum subdermal needle electrodes (model F-E2, Astro-Med Inc, West Warwick, RI, USA) were used as the ground and reference electrodes. The ground electrode was placed subcutaneously on the apex of the occiput, and the reference electrode was placed at approximately 0.3-0.5 cm lateral to the lateral canthus of the eye. Proparacaine hydrochloride (Alcaine, Alcon, Vilvoorde, Belgium) eye drops were applied to the cornea as a topical anesthetic before placing the corneal electrode.

The pigeons were dark adapted for 20 minutes before the scotopic mixed rod and cone response was obtained at a stimulus intensity of 3.0 cd s/m^2. Four responses from flashes with an interval of 17 seconds were averaged. The pigeons were then light adapted for 10 minutes using a background light of 30 cd/m^2, and the photopic cone and flicker responses were recorded. The flash presented at a stimulus intensity of 3.0 cd s/m^2 were delivered with a stimulus frequency of 2 Hz for the photopic cone and 27.78 Hz for the flicker. The pigeons were monitored for signs of systemic side effects from the topical application of rocuronium bromide throughout the ERG procedure until the next day.

3. Statistical analysis

The data obtained for the scotopic mixed rod and cone and photopic cone responses were the a- and b-wave implicit times and amplitudes. For the photopic flicker response, the data obtained were the peak implicit time and amplitude. Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). A Student's *t*-test was performed to compare the data (a- and b-wave implicit times and amplitudes) between the groups, except for the data of a-wave implicit time from photopic cone response, which was analyzed using the Wilcoxon rank-sum test (Mann-Whitney U test). A P-value of < 0.05 was considered statistically significant.

Results

The general ophthalmic examinations of the experimental pigeons were all normal. No retinal lesions were observed on the funduscopic examination. IOP was ranged from 9 to 14 mmHg. The pigeon's pupil size without the application of rocuronium bromide were 3.05 ± 0.24 mm in diameter. On the second set of ERG recordings, after the dark adaptation stage, the pupil was checked under dim red light (not directly shone to the eye) to ensure successful pharmacological pupil dilation. However, pupil measurement was only done after the ERG recording had finished as not to interfere with the time/duration and the effects of the sedation, dark adaptation, and mydriasis. The pupil diameter after the second ERG recordings were 4.46 ± 0.35 mm. Signs of systemic toxicity typical of those from the application of neuromuscular blocking agent such as neck and limb muscle paralysis were not observed from the topical application of rocuronium bromide.

In the scotopic mixed rod and cone response and the photopic cone response, the ERG of the domestic pigeons showed a negative deflection followed by a positive ascending slope, which were identified as the a- and b-waves, respectively (Fig. 3A-D). The photopic flicker introduced at a stimulus intensity of 3.0 cd s/m^2, and a frequency of 27.78 Hz showed a series of waves following each light stimulus (Fig. 3E and 3F).

During the scotopic mixed rod and cone response, the implicit times obtained during the ERG of the control group were 11.67 ± 2.17 and 38.15 ± 9.35 ms for the a- and b-waves, respectively. With the induction of mydriasis on the treatment group, the implicit time for the a- and b-waves were 12.82 ± 0.64 and 31.92 ± 2.60 ms, respectively. The ERG amplitudes obtained in the control group for the a- and b-waves were 46.80 ± 25.93 and 145.52 ± 56.92 μV compared to 100.30 ± 62.13 and 206.81 ± 132.41 μV in the treatment group, respectively (Table 3). There were no statistically significant differences of the a- and b-wave implicit times and amplitudes in the scotopic mixed rod and cone ERG of the domestic pigeons between the control and the treatment groups.

However, in the photopic condition, statistically significant differences were observed in the a- and b-wave amplitudes of the photopic cone response in which the amplitudes of both the a- and b-waves were higher in the ERG of the treatment group (P = 0.045 and P = 0.032, respectively). The a- and b-waves of the photopic cone ERG amplitudes in the treatment group were 63.83 ± 32.33 and 191.75 ± 94.46 μV, which were significantly higher than 46.15 ± 27.60 and 116.76 ± 70.65 μV in the control group, respectively (Table 4). In the photopic flicker response, the flicker amplitude was also significantly higher in the treatment group (76.23 ± 48.56 μV) than the control group (42.18 ± 31.18 μV; P = 0.044; Table 5). As for the implicit times, there were no statistically significant differences between the two groups in both the photopic cone and photopic flicker responses. The implicit times of the a- and b-waves obtained during the photopic cone ERG in the control group were 11.60 ± 2.07 and 30.37 ± 6.32 ms compared to 10.98 ± 0.73 and 27.55 ± 3.23 ms in the treatment group, respectively (Table 4). The implicit time for photopic flicker in the control group was 12.28 ± 1.63 ms compared to 11.95 ± 1.58 ms in the treatment group (Table 5).

Oscillatory potentials (OPs) were observed in the ascending slope between the a- and b-waves of the scotopic mixed rod and cone response and photopic cone response (Fig. 3A-D). These oscillatory potentials were composed of up to three peaks in this study.

Table 3. Descriptive statistics of the scotopic mixed rod and cone electroretinography in pigeons with and without rocuronium bromide to induce mydriasis

Scotopic mixed rod and cone responses				
Group	**Implicit time (ms)**		**Amplitude (µV)**	
	a-wave	**b-wave**	**a-wave**	**b-wave**
Control	11.67 ± 2.17*	38.15 ± 9.35	46.80 ± 25.93	145.52 ± 56.92
Treatment	12.82 ± 0.64	31.92 ± 2.60	100.30 ± 62.13	206.81 ± 132.41

*Mean ± SD.

Table 4. Descriptive statistics of photopic cone electroretinography in pigeons with and without rocuronium bromide to induce mydriasis

Photopic cone responses				
Group	Implicit time (ms)		Amplitude (µV)	
	a-wave	b-wave	a-wave	b-wave
Control	11.60 ± 2.07[*]	30.37 ± 6.32	46.15 ± 27.60[a]	116.76 ± 70.65[a]
Treatment	10.98 ± 0.73	27.55 ± 3.23	63.83 ± 32.33[b]	191.75 ± 94.46[b]

Wilcoxon rank-sum test (Mann-Whitney U test) was used to compare the a-wave implicit times between control and treatment groups. The b-wave implicit time and the a- and b-wave amplitudes were analyzed using Student's *t*-test.

[a,b] Different superscript letters indicate significant differences in the same column at a P-value of < 0.05.

[*]Mean ± SD.

Table 5. Descriptive statistics of photopic flicker electroretinography in pigeons with and without rocuronium bromide to induce mydriasis

Mydriasis	**Photopic flicker responses**	
	Implicit time (ms)	**Amplitude (μV)**
Control	12.28 ± 1.63*	42.18 ± 31.18[a]
Treatment	11.95 ± 1.58	76.23 ± 48.56[b]

Student's *t*-test were used to compared the implicit times and amplitudes between the control and treatment groups.

[a,b] Different superscript letters indicate significant differences in the same column at a *P*-value of < 0.05.

*Mean ± SD.

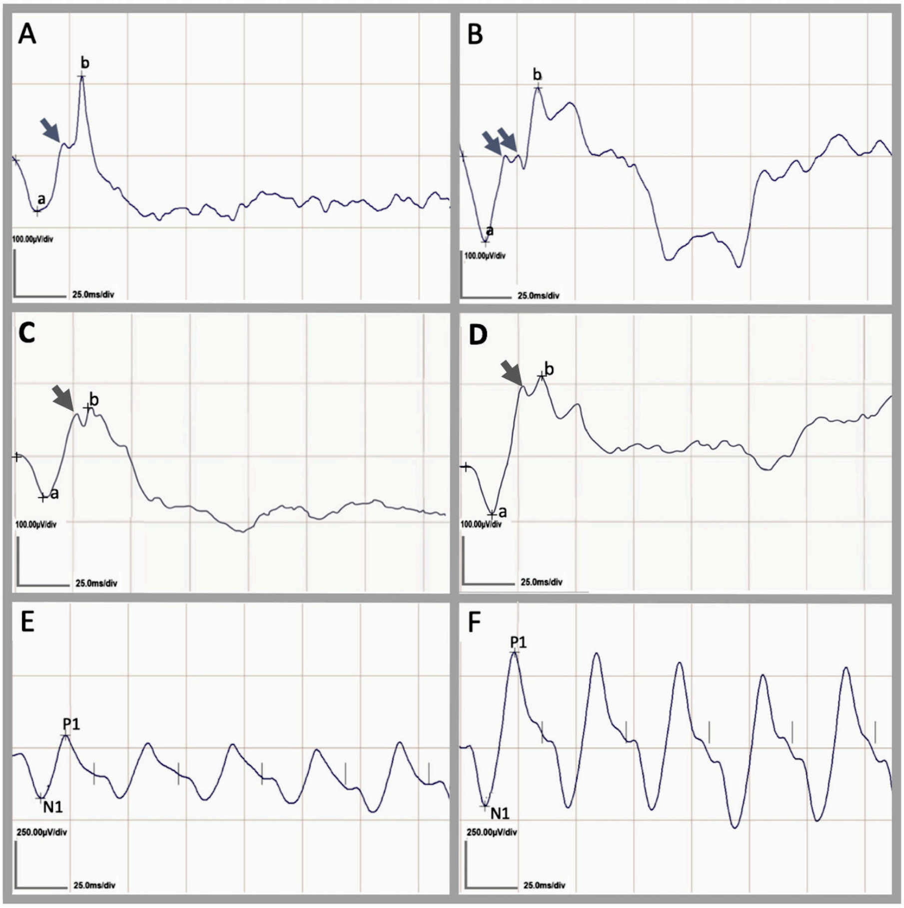

Figure 1. Representative electroretinography of domestic pigeons with and without rocuronium bromide to induce mydriasis. The scotopic mixed rod and cone response: (A) without rocuronium bromide, and (B) with rocuronium bromide to induce mydriasis. The photopic cone response: (C) without rocuronium bromide, and (D) with rocuronium bromide. The photopic flicker response: (E) without rocuronium bromide, and (E) with rocuronium bromide. The letters a and b denote the a- and b-waves, respectively. N1 denotes the implicit time, and P1 denotes the amplitude of the photopic flicker response. The bar in between the waves of photopic flicker represents the introduction of light stimulus except for the first stimulus (on E and F). Oscillatory potentials (OP) were observed during on the ascending slope between the a- and b-waves (arrows; A, B, C and D).

Discussion

ERG is generated by the summed activity of all retinal cells that consist of overlapping positive and negative component potentials originated from different stages of retinal processing (Frishman, 2018). The initial negative wave in response to light stimuli is known as the a-wave, followed by the positive peak known as the b-wave (Fig. 3; Ekesten, 2013; Frishman, 2018; Pasmanter and Petersen-Jones, 2020). Under the scotopic condition, the main generator of the a-wave to a low intensity stimulus is the rod photoreceptors, as the cone photoreceptors are not as sensitive to light (Pasmanter and Petersen-Jones, 2020). However, in this study, the stimulus presented during the scotopic condition was strong enough to elicit a response from both photoreceptor types (Figs. 3A and 3B). This protocol was based on the ERG guideline for dogs in which the bright stimulus of 3.0 cd s/m^2 was designed to elicit rod and cone responses after the animal underwent a dark adaptation stage (Ekesten *et al*., 2013). As for the photopic condition, the a-wave was driven by cone photoreceptors because the rod photoreceptor was suppressed by the light adaptation stage (Figs. 3C and 3D; Pasmanter and Petersen-Jones, 2020). Aside from the photoreceptors, the bipolar cells, amacrine, and ganglion cells also contribute to the a-wave (Frishman, 2018; Pasmanter and Petersen-Jones, 2020). In both dark- and light-adapted conditions, the main generator of the b-wave is the ON-bipolar cells (Frishman, 2018).

During ERG, the contribution from each retinal cell generally increases with the area of the retina stimulated as the number of cells, and hence the total extracellular current is increased (Frishman, 2018). Mydriasis allows more photons to enter the eye, resulting in increased retinal illumination (Ofri, 2013). Therefore, it could be expected that ERG recorded in a mydriatic pupil (treatment group) would result in a higher value as seen in both the a- and b-wave amplitudes of the photopic cone and photopic flicker response in this study (Tables 4 and 5). Performing ERG on miotic pupils, not only could potentially reduce the amplitude, but might also increase the implicit time (Ekesten, 2013). This lower amplitude and attenuated implicit time might be mistaken for retinal disease, which was why ERG was recommended to be performed on eyes with mydriatic pupils (Ekesten, 2013). In the present study, no differences were observed between the amplitudes of the mydriatic and non-mydriatic pupils on the scotopic condition and only a lower amplitude was observed in the photopic ERG of the control group compared to the treatment group (in both photopic cone and photopic flicker response). Furthermore, no differences were observed in the implicit time from all ERG recordings.

The lack of a significant difference between both groups in the implicit times and amplitudes of our scotopic results might be caused by the possibility that in the dark-adapted stage, the pigeon's pupil underwent mydriasis naturally. Therefore, the scotopic ERG between both groups was not significantly different as both pupils were dilated. In contrast, the photopic protocols were preceded by a light adaptation stage in which the pigeon's eye was illuminated for 10 minutes with a background light of 30 cd/m^2. This light adaptation procedure was designed to suppress the function of rod photoreceptors but, at the same time, would also constrict the pupil in the control group in the absence of rocuronium bromide to keep the pupil dilated. Study in human comparing ERG between eyes with dilated pupil and non-dilated pupil observed that in scotopic condition the non-dilated pupil underwent some degree of natural dilation but never achieved the diameter resulted from the application of mydriatics (Gagne *et al*., 2010). The human study also showed significant retinal sensitivity reduction in eye without mydriatics (Gagne *et al*., 2010). Differed with the present study, the b-wave of the scotopic ERG in human showed significant different between the non-dilated and dilated eye (Gagne *et al*., 2010). However, upon eliminating one seemingly misplaced data, the different between both conditions became insignificant (Gagne *et al*., 2010) which in agreement with the present study.

In human, pupil dilates significantly less in elderly compared to the young (Gagne *et al*., 2010). ERG study in pigeon with a wide range of age reported no systematic differences in pupil diameter between the old and young pigeon (Porciatti *et al*., 1991). The mydriatic agent used in the present study was rocuronium bromide, a neuromuscular blocking agent with an affinity for acetylcholine (ACh) receptors, which acts as an ACh competitor in the neuromuscular junction of the bird's iris striated muscle, resulting in mydriasis (Keegan, 2015). Previous ERG study in pigeon utilized topical d-tubocurarine chloride to induce mydriasis (Bagnoli *et al*., 1985; Porciatti *et al*., 1991) and although d-tubocurarine chloride was reported as unreliable in inducing mydriasis (Verchueren and Lumeij, 1991), the pupil diameter achieved in the previous study was 4.6 – 5.0 mm in diameter (Porciatti *et al*., 1991). In the present study, the degree of mydriasis achieved by rocuronium bromide was only measured after the ERG recording had ended. This was done to avoid the delay on ERG recording and therefore waste the effect of sedation and mydriasis as well as potentially disrupt the dark-adapted retina. The pupil diameter after the ERG procedure ranged from 4.00 to 5.00 (4.46 ± 0.35) mm. Since mydriasis produced by rocuronium bromide in pigeon was observed after 5 minutes and lasting for two hours (Susanti *et al.*, 2021), the mydriatic effect after 20 minutes of dark adaptation should be optimal to facilitate ERG recording.

The current guideline for ERG in dogs advocates 20 minutes of dark adaptation stage before the scotopic mixed rod and cone response to allow for the rod photoreceptor to gain its sensitivity (Ekesten *et al.*, 2013). The dark adaptation stage employed on the previous report of ERG performed on birds had been varied from 4 minutes (Hendrix *et al.*, 2004), 5 minutes (Seruca *et al.*, 2012), 10 minutes (Kuhn *et al.*, 2014) and 20 minutes (Labelle *et al.*, 2012; Susanti *et al.*, 2019). However, since the protocol and settings of ERG performed were all different, it was hard to compare the results for all the study. In the present study, the dark adaptation was done for a minimum of 20 minutes before each scotopic ERG recording as suggested by the current ERG guideline (Ekesten *et al.*, 2013). In the comprehensive ERG testing, on the protocol for both dogs and human, a special test designed to evaluate rod function is performed during the dark adaptation stage called the rod-driven test (Ekesten *et al.*, 2013). In this test, a series of flashes with a low intensity is introduced every 5 minutes during at least 20 minutes of dark adaptation and (ideally) performed until the b-wave amplitude produced reaches a stable plateau (Ekesten *et al.*, 2013). However, this rod-driven response was not performed in this study as this test would require a prolong handling of the pigeon during the dark adaptation.

As discussed above, the main reason for mydriatic induction during ERG was to ensure equal distribution of the light stimulus on the retina in order to obtain a maximum amplitude and avoid prolonged implicit time (Ekesten, 2013). However, study in mouse anesthetized with ketamine and xylazine, showed that certain combination of mydriatics could also directly affecting the electrogenesis on the retina (Mojumder and Wensel, 2010). The combination of atropine and phenylephrine was reported to produce a significantly higher b-wave amplitude when compared to atropine alone or without the use of mydriatics in ketamine and xylazine anesthetized mouse despite the similar pupil size (Mojumder and Wensel, 2010). The atropine and phenylephrine combination were thought to affect the signaling of the amacrine and ganglion cell circuit in the retina resulting in a higher b-wave amplitude (Mojumder and Wensel, 2010). Considering that the amacrine cell of the pigeon's retina also possessed ACh receptors (White *et al*., 1990) and rocuronium bromide binds to the ACh receptors, further study is needed to determine if rocuronium bromide could directly affect the electrogenesis on the pigeon's retina.

The a-wave and b-wave are the two waves most commonly used to evaluate retinal diseases in clinical setting (Freeman *et al*., 2013). It is well-known that several technical aspects can influence the amplitudes and implicit times of the a- and b-waves of the ERG recording, for example, sedation and anesthesia (Ekesten, 2013; Ekesten *et al*., 2013; Freeman *et al*., 2013). However, these effects are not always observed in pairs. For example, medetomidine and its isomer, dexmedetomidine, have consistently been reported to prolong the implicit time of both a- and b-waves and also lower its amplitudes when compared to awake dogs

(Norman *et al.*, 2008; Freeman *et al.*, 2013). In contrast, general anesthesia was found to lower both a- and b-wave amplitudes but only prolong the a-wave implicit time (Freeman *et al.*, 2013). A comparison of alfaxalone and medetomidine sedation during ERG recording of pigeons also only differed in the a-wave implicit time and b-wave amplitude (Susanti *et al.*, 2019). In the present study, although the non-mydriatic pupils of the control group resulted in lower amplitudes of both photopic cone and photopic flicker, none of the implicit times were prolonged.

Alfaxalone was used to induce sedation in this study as it was reported to have less effect on pigeon's ERG compared with alpha-2 adrenergic receptor agonists (Susanti *et al.*, 2019). Here, the level of sedation obtained was adequate to facilitate the handling of pigeons throughout the procedure with minimal stress, but there is an inherit possibility that should alfaxalone has any suppression effects to the ERG recording, the early recording might have had been affected more by the sedation compared to the later. However, previous report on pigeons showed the b-wave of the photopic ERG recorded under alfaxalone sedation to be higher than that of medetomidine sedation (Susanti *et al.*, 2019), the later widely known to depress the ERG recordings (Freeman *et al.*, 2013). The use of anesthesia during ERG recording has always been recommended as it will help to minimize the noise and artifacts from involuntary muscles, especially for a long ERG session (Ekesten *et al.*, 2013; Freeman *et al.*, 2013). As for a shorter ERG protocols, performing ERG without chemical restrain is considered acceptable, with no significant differences in the low- or high-frequency noise levels found between sedated and anesthetized dogs (Freeman *et al.*, 2013). ERG recordings of Hispaniolan Amazon parrots (*Amazona ventralis*) and two species of owl were performed under general anesthesia using isoflurane (Hendrix and Sims, 2004; Seruca *et al*., 2012), and sevoflurane was used for ERG recording in bald eagle (*Haliaeetus leucocephalus*) (Kuhn *et al*., 2014). While mydriasis was not induced in those studies, isoflurane was known to cause mydriasis in birds (Longley, 2008), which might be beneficial in retinal illumination when mydriasis is not utilized during ERG recording.

In the present study, OPs ranged from one to three peaks at the ascending slopes between the a- and b-waves were observed in both pupil conditions. OPs are a series of wavelets superimposed mainly on the ascending limb of the b-wave whose number and appearance vary between individuals and species (Wachtmeister, 1998; Hendrix and Sims, 2004; Ekesten, 2013). Three to five peaks are usually observed in the dark-adapted response in cats and dogs (Ekesten, 2013). In Hispaniolan Amazon parrots, OPs were consisted of three to four peaks (Hendrix and Sims, 2004). Although special adaptation levels and filtering techniques are required to record OPs, sometimes they are seen during the recoding of a- and b-waves (Wachtmeister, 1998). In this study, photopic flicker was introduced at a frequency of 27.78 Hz even though pigeons have the highest flicker-fusion frequency reported among vertebrates, of 143 Hz (Lisney *et al*., 2011). Performing photopic flicker ERG in non-mydriatic pupils (control group) resulted in a lower amplitude than photopic flicker in mydriatic pupils (treatment group).

Conclusions

In conclusion, rocuronium bromide provide an adequate mydriasis on pigeon underwent ERG resulting in higher amplitudes of both photopic cone and photopic flicker ERG. However, as pigeon pupil naturally underwent mydriasis in scotopic condition, there were no significant different observed during the scotopic mixed rod and cone ERG.

GENERAL CONCLUSIONS

Topical rocuronium bromide produced moderate mydriasis in pigeons other than bull eye in this study without significant side effects except a transient lower eyelid paresis in 5/8 pigeons. This lack of efficacy in pigeon, despite being reported to be 100% effective in other species of birds was probably caused by different pigmentation on the different iris color in pigeons. However, although the mydriasis produced by rocuronium bromide in pigeons was only moderately effective in this study, the degree of mydriasis achieved could facilitate advanced ophthalmic examinations such as ERG.

Comparison between scotopic ERG in domestic pigeon with and without the induction of mydriasis showed no differences in the implicit times and amplitudes. This was most likely because in the scotopic condition, pigeon's pupil underwent natural dilation and therefore resulting in a relatively same amount of light entering the eyes on both mydriatic and non-mydriatic eyes. However, in both photopic cone and photopic flicker ERG, the amplitudes in the mydriatic pupil were higher than that in the non-mydriatic pupil. As for the implicit times, there were no differences observed in both photopic cone and photopic flicker ERG. Thus, this study suggested that performing ERG without the induction of mydriasis resulted in significantly lower amplitudes in photopic condition.

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국 문 초 록

비둘기(*Columba livia*)에서 Rocuronium Bromide의 점안이 산동 및 망막전위도에 미치는 영향

지도교수 서 강 문

Lina Susanti

서울대학교 대학원

수의학과 임상수의학 전공

본 연구는 rocuronium bromide의 점안이 비둘기(*Columba livia*)의 산동 및 망막전위도(electroretinography)에 미치는 영향을 평가하기 위하여 실시되었다.

제1장에서는 신경근 차단제인 rocuronium bromide가 비둘기의 산동에 미치는 영향을 2 단계에 걸쳐 평가하였다. 첫번째 단계에서는 rocuronium bromide (0.20 mg/20 μL)를 8마리의 비둘기

의 우안(OD)에 점안하였다. 첫번째 단계에서 사용된 비둘기의 홍채 색은 gravel, pearl, bull eye로 구성되었다. 동공의 직경 및 동공 빛반사(PLR)는 약물 점안 전(T0), 점안 5분 후(T05), 점안 10분 후(T10), 그리고 이후부터는 점안 160분 후(T160)까지 10분마다 측정하였다. 동공 직경은 mm 단위로 측정되었으며 PLR은 점수 체계를 이용하여 평가하였다. 첫번째 단계에서 rocuronium bromide는 8마리 중 6마리의 비둘기에서 산동을 유발하였다. 동공 직경은 T30에서 최대였으며 평균 4.62 ± 0.13 mm로 관찰되었다. Rocuronium bromide를 점안한 눈의 T0에서의 동공 직경은 T05-T120에서의 동공 직경과 유의미한 차이를 보였다($P < 0.05$). 또한 rocuronium bromide을 점안한 눈과 점안하지 않은 눈의 동공 직경은 T05-T120에서 각각 유의미한 차이가 확인 되었다($P < 0.05$). T10부터 T90까지 PLR은 없었다. 두번째 단계에서는 4마리 비둘기의 양안(OU), 총 8개의 안구에 첫번째 단계와 동일한 양의 rocuronium bromide을 10분 간격으로 2회 점안하였다. 이 단계에서 사용된 모든 비둘기의 홍채 색은 bull eye였다. 동공 직경 및 PLR의 평가 방법과 측정 시간은 첫번째 단계와 동일하였다. 평가된 8개의 눈 중 2개의 눈에서만 산동이 확인되었다. 이를 통하여 rocuronium bromide는 홍채 색이 bull eye가 아닌 비둘기에서 산동을 일으킬 수 있음을 알 수 있었다. 일시적인 눈꺼풀의 경

련을 제외하고는 실험 중 유의적인 부작용은 관찰되지 않았다.

제2장에서는 rocuronium bromide의 점안이 비둘기의 ERG에 미치는 영향을 평가하였다. 9마리의 성체 비둘기에서 rocuronium bromide의 점안을 통하여 유도된 산동 상태와, 산동을 하지 않은 상태에서 각각 ERG를 실시하였으며, scotopic mixed rod and cone, photopic cone 및 photopic flicker 등을 측정하여 그 결과를 비교하였다. Scotopic mixed rod and cone ERG에서는 산동 유무에 따른 유의적인 차이가 확인되지 않았다. 하지만 photopic ERG에서는 산동 유무에 따라 photopic cone과 photopic flicker의 amplitude 모두에서 유의적인 차이가 확인되었다. Photopic ERG의 산동상태에서의 amplitude가 유의적으로 더 높았다.

본 연구 결과는 산동 상태에서, rocuronium bromide의 점안은 bull eye 이외의 홍채 색을 가진 비둘기에서 심각한 부작용 없이 안정적으로 산동을 유발하였으며, rocuronium bromide에 의해 유발된 산동으로 비둘기에서 ERG 같은 정밀 안검사가 가능함을 알 수 있었다. 또한 비둘기에서 rocuronium bromide에 의해 산동 된 눈의 photopic ERG의 amplitude는 산동을 하지 않은 눈에 비하여 유의미하게 더 높았음을 알 수 있었다. 따라서 비둘기에서 일반적

인 안검사 및 망막전위도 검사 시 산동제로서 rocuronium bromide를 사용할 수 있을 것으로 사료된다.

주요어: *Columba livia*, 망막전위도, 비둘기, 산동, rocuronium bromide.

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