คุณลักษณะคลื่นไฟฟ้าจอตาแบบแสงวาบตามอนุกรมความเข้มแสงในสุนัขต้อกระจกเหตุเบาหวาน

นางสาวฐาปนีย์ ลีพรหมรัตน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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ปีการศึกษา 2557

CHARACTERIZATIONS OF FLASH INTENSITY-SERIES ELECTRORETINOGRAPHY IN DOGS WITH DIABETIC CATARACT

Miss Thapanee Leepromrath

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Veterinary Surgery
Department of Veterinary Surgery
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ฐาปนีย์ ลีพรหมรัตน์ : คุณลักษณะคลื่นไฟฟ้าจอตาแบบแสงวาบตามอนุกรมความเข้มแสงในสุนัขต้อกระจกเหตุเบาหวาน (CHARACTERIZATIONS OF FLASH INTENSITY-SERIES ELECTRORETINOGRAPHY IN DOGS WITH DIABETIC CATARACT) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. สพ.ญ. ดร.นลินี ตันติวนิช, 52 หน้า.

เบาหวานเป็นความผิดปกติของฮอร์โมนที่พบบ่อยทั้งในคนและในสุนัข ภาวะจอตาเหตุเบาหวานเป็นสาเหตุให้ผู้ป่วยเบาหวานมีการมองเห็นที่เปลี่ยนแปลงไป สุนัขป่วยเบาหวานมักสูญเสียการมองเห็นจากต้อกระจกเหตุเบาหวานทั้งสองตามีการพัฒนาอย่างรวดเร็ว การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาคุณลักษณะของคลื่นไฟฟ้าจอตาแบบแสงวาบสีขาวตามอนุกรมความเข้มแสงในสุนัขป่วยด้วยต้อกระจกเหตุเบาหวาน โดยศึกษาในสุนัขต้อกระจกระยะสุดท้ายทั้งหมด 50 ตัว แบ่งเป็นกลุ่มสุนัขต้อกระจกเหตุเบาหวาน 25 ตัว (46 ตา) และกลุ่มสุนัขที่เป็นต้อกระจกปราศจากเบาหวาน 25 ตัว (45 ตา) สุนัขได้รับการวัดคลื่นไฟฟ้าจอตาทั้งที่มืดและที่สว่างภายใต้ภาวะสลบแบบทั้งตัว ใช้เลนส์สัมผัสชนิดเจทเป็นขั้วไฟฟ้าบวกและใช้เข็มเป็นขั้วไฟฟ้าลบ ผลการศึกษาพบว่าสุนัขต้อกระจกเหตุเบาหวานมีค่าเฉลี่ยของทั้งคลื่น a และคลื่น b ลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) ในทุกความเข้มแสง พบลักษณะคลื่นไฟฟ้าจอตาแบบลบในบางตาที่มีภาวะเบาหวาน ค่าเฉลี่ยของคลื่น oscillatory potential ในสุนัขเบาหวานมีการเปลี่ยนแปลงไปอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนั้นยังพบว่าค่าเฉลี่ยความสูงของคลื่น flicker ก็ลดลงอย่างมีนัยสำคัญทางสถิติ ($p<0.05$) เช่นกัน การที่ความสูงของคลื่นไฟฟ้าจอตาลดลงอย่างมีนัยสำคัญในสุนัขเบาหวานนั้นนอกจากจะบ่งชี้ว่าภาวะจอตาขาดเลือดจากเบาหวานมีการส่งกระแสประสาทลดลงแล้ว ยังบอกถึงความต้องการสันดาปภายในจอตาอีกด้วย ลักษณะคลื่นไฟฟ้าจอตาแบบลบที่พบในสุนัขเบาหวานอาจมีความเกี่ยวเนื่องกับการอุดตันของเส้นเลือดจอตาส่วนกลางที่ทำให้จอตาชั้นในขาดเลือดอย่างรุนแรง ทั้งนี้การที่คลื่น oscillatory potential มีการเปลี่ยนแปลงไป แสดงให้เห็นถึงความเสื่อมในระยะแรกของเซลล์สองขั้ว และเซลล์ปมประสาทในจอตาชั้นใน ดังนั้นการตรวจคลื่นไฟฟ้าจอตาแบบแสงวาบจึงเป็นเครื่องมือวินิจฉัยที่จำเป็นและมีความไวต่อการประเมินการทำงานของจอตาในสุนัขเบาหวานตั้งแต่ระยะแรกที่ผิดปกติก่อนพิจารณาทำศัลยกรรมแก้ไขต้อกระจกเหตุเบาหวาน

ภาควิชา ศัลยศาสตร์ ลายมือชื่อนิสิต .....

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5575308631 : MAJOR VETERINARY SURGERY
KEYWORDS: CATARACT / DIABETIC MELLITUS / DOGS / ELECTRORETINOGRAPHY / RETINA

THAPANEE LEEPROMRATH: CHARACTERIZATIONS OF FLASH INTENSITY-SERIES ELECTRORETINOGRAPHY IN DOGS WITH DIABETIC CATARACT. ADVISOR: ASST. PROF. NALINEE TUNTIVANICH, D.V.M., Ph.D., 52 pp.

Diabetes mellitus is the common endocrine disease in humans and in dogs. Visual impairment in diabetic patients is caused by diabetic retinopathy. Dogs with diabetic mellitus rapidly develops bilateral cataract, leading to blindness. This study was to see the electroretinographic characteristics in response to the full-field intensity-series white flash electroretinography in diabetic dogs. Fifty dogs (46 diabetic eyes and 45 non-diabetic eyes) with late stage cataract were included in this study. Under general anesthesia, scotopic and photopic electroretinographic responses were recorded using JET lens as a corneal contact lens electrode and a needle as a reference electrode. In diabetic dogs, mean a- and b-wave responses were significantly reduced in amplitude ($p<0.05$) at all light stimuli. Negative ERG responses were found in some diabetic eyes. Mean oscillatory potential in diabetic dogs was significantly altered ($p<0.05$). Significant reduction of the mean flicker amplitude was also observed ($p<0.05$). In diabetic dogs, significant decrease of electroretinographic amplitudes not only is an indicative of reduction of neuronal signal, but also metabolic demand in ischemic retina. Negative ERG responses that were found in some canine diabetic eyes may possibly be related to severe inner retinal ischemia from central retinal occlusion. Changes of the oscillatory potential reflects early impairment of bipolar and ganglion cells. Flash electroretinography is an essential and sensitive diagnostic tool for early assessment of retinal function in diabetic dogs prior to cataract surgery consideration.

Department: Veterinary Surgery Student's Signature .....
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LIST OF ABBREVIATIONS

ALP	=	alkaline phosphatase
BUN	=	blood urea nitrogen
cd/m^2	=	candela per square meter
cds/m^2	=	candela second per square meter
CSNB	=	congenital stationary night blindness
DKA	=	diabetic ketoacidosis
dl	=	deciliter
DM	=	diabetes mellitus
DR	=	diabetic retinopathy
dUTP	=	deoxyuridine triphosphate
ERG	=	electroretinography
GABA	=	gamma-aminobutyric acid
GCL	=	ganglion cell layer
h	=	hour
IDDM	=	insulin-dependent diabetes mellitus
ILM	=	inner limiting membrane
IPL	=	inner plexiform layer
INL	=	inner nuclear layer
ISCEV	=	International Society for Clinical Electrophysiology
kg	=	kilogram
mg	=	milligram
ml	=	mililitre
ms	=	millisecond
Na^+/K^+-ATPase	=	sodium-potassium adenosine triphosphatase
NIDM	=	noninsulin-dependent diabetes mellitus
NPDR	=	non-proliferative diabetic retinopathy
NFL	=	nerve fiber layer

OPs	=	oscillatory potentials
OLM	=	outer limiting membrane
ONL	=	outer nuclear layer
OPL	=	outer plexiform layer
OCT	=	optical coherence tomography
PDR	=	proliferative diabetic retinopathy
PRA	=	progressive retinal atrophy
RBC	=	red blood cell
RPE	=	retinal pigment epithelium
RIAs	=	radioimmunoassay
SARDs	=	sudden acquired retinal degeneration
SGPT	=	serum glutamic-pyruvic transaminase
μV	=	microvolt
μmol	=	micromole
VEGF	=	vascular endothelial growth factor
WBC	=	white blood cell

Chapter 1
Introduction

Importance and Rationale.

Diabetes mellitus (DM) is characterized by deficiency of insulin involved by several pathophysiological mechanisms (Davison, 2010). Many complications have been associated with DM; such as diabetic retinopathy (Safatle et al., 2010) peripheral neuropathy (Morgan et al., 2008), retinal hemorrhage (Landry et al., 2004), diabetic cataract and spontaneous lens capsule rupture (Wilkie et al., 2006). Diabetic patients could have decreased visual acuity or sudden loss of vision (Chistiakov, 2011).

Diabetic retinopathy (DR) is one of the major complications in humans with DM. Out of 93 million people from 35 countries investigated, the prevalence of DR was 34.6% (Yau et al., 2012). Fundus examination revealed macular edema, microaneurysm and proliferative retinal blood vessels that lead to retinal hemorrhage and blindness in human (Chistiakov, 2011). Visual deficit could occur during an early stage of the disease when abnormalities of retinal blood vessels had not yet been detected by fundus photography (Tzekov and Arden 1999).

Electroretinography (ERG) is the sensible diagnostic tool (Lee et al., 2009) to assess retinal function before ophthalmic lesions of DR have developed (Shirao and Kawasaki, 1998). To attain standardization of ERG recording, ERG protocol was recommended by the International Society for Clinical Electrophysiology of Vision (ISCEV) in humans (Marmor et al., 2009) and the European College of Veterinary Ophthalmologist (ECVO) in animals (Ekesten 2013). Deficit of b-wave amplitude and

oscillatory potential response were reported in diabetic patients (Hiraiwa et al., 2002, Gualtieri et al., 2013), reflecting substantial damage in the inner retina.

Unlike humans, diabetic dogs rapidly developed bilateral cataracts (Catchpole et al., 2005). Marked opacification of lenses not only causes visual impairment in dogs but also obscures routine fundus examination (Enl et al., 2009). Documentation of fundus alteration in dogs was therefore limited. 21% of diabetic dogs with diabetic cataract undergone for phacoemulsification had retinal hemorrhage and microaneurysm (Landry et al., 2004). Investigation of retinal function by ERG prior to cataract surgery will not only provide more information of retinal injury, but also offer prognosis of visual return after cataract surgery (Ratanapakorn et al., 2010).

Objectives of study

To characterize a full-field intensity-series of white flash electroretinography in dogs with late stage diabetic cataract

Research question

Can electrical changes in the retina of canine diabetic cataract be detected with the use of a full-field intensity-series white flash electroretinography?

Chapter 2

Literature review

Diabetes mellitus

Diabetes mellitus (DM) is an endocrine disorder that pancreatic beta cells fail to secrete adequate insulin production to control blood glucose level. Base on function of beta cells, DM is divided into two types (Nelson, 2005); insulin-dependent and noninsulin-dependent DM. Insulin-dependent diabetes mellitus (IDDM) is the condition that pancreatic beta cells are absence. Insulin production is inadequate so created hypoinsulinemia. It can be congenital or acquired disorder that is curative by insulin replacement (Catchpole et al., 2005). Noninsulin-dependent diabetes mellitus (NIDM), on the other hand, is not related to productive ability. Insulin is well secreted but cannot properly function. Insulin resistance then occurs; hyperglycemia associated with hyperinsulinemia. NIDM frequently occurs in humans because of obesity (Dabelea et al., 2007). In dogs, if hyperglycemia is prolonged in NIDM, IDDM will occur due to a permanent secondary loss of beta cells (Catchpole, 2007).

IDDM is common in dogs (Catchpole et al., 2005). It is due to that fact that dogs usually have immune-mediated pancreatic beta cells destruction (Rucinsky et al., 2010). Breeds predisposing to DM were Beagle, Australian terrier, Samoyed, etc, all of which were diagnosed with DM at age less than 12 months (Catchpole et al., 2005; Catchpole et al., 2008). Intact females had 2.6 times higher risk of DM compared to males (Fall et al., 2007). Excessive corticosteroid induces hyperglycemia by stimulating gluconeogenesis (McMahon et al., 1988). Prolonged or repeated progesterone exposure stimulated peripheral resistance of insulin leading to DM in dogs (Rosmond, 2003). Moreover, degeneration of beta cells could be triggered by other risk factors such as pancreatitis, obesity and some medications (Nelson, 2005).

Polydipsia and polyuria have been known as typical clinical signs of DM. When blood glucose level exceeds renal tubular threshold, glucose is excreted into urine, resulting in glucosuria. Glucosuria was diagnosed when blood glucose level was above 200mg/dl (Rucinsky et al., 2010). When level of insulin is insufficient to activate hypothalamic satiety center, other atypical clinical signs such as diabetic ketoacidosis, polyphagia (Davison, 2012), weakness, weight loss (Catchpole et al., 2005; Davison, 2012), rough hair, dehydration, lens opacity, abdominal cramp, increased sensitivity to infection of skin or urinary bladder, ketone sweet odor from mouth and ketosis could be found. Heart disease and renal disease could shortly be associated (Rucinsky et al., 2010).

In addition to clinical signs, DM can be diagnosed by investigation of fasting blood glucose level after 8 hours fasting period. Fasting blood glucose level in dogs with DM is above 180 mg/dl. Meanwhile it is 70-90 mg/dl in normal (Rucinsky et al., 2010). Glucosuria can be diagnosed by urinalysis (Catchpole et al., 2005). Investigation of serum fructosamine is another routine test to assess level of glycosylated hemoglobin, guiding to average blood glucose level at approximately 2-3 months earlier (Davison, 2012). In dogs that long-term hyperglycemia is suspicious but blood glucose level is uncertain, DM can be confirmed by measurement of glycated blood proteins, such as fructosamine or glycosylated haemoglobin. While serum fructosamine concentration is related to the average blood glucose concentration over the preceding 1-2 weeks, level of glycosylated haemoglobin reflects the average blood glucose concentrations over the preceding 2-3 months (Jansen, 1995). If clinical signs of DM are not well recognized, investigation of insulin via radioimmunoassays has become available (Catchpole et al., 2005).

In humans, oral medication such as sulfonylurea (Abraira et al., 1998) has been prescribed in order to increase insulin production. Since dogs have different type of DM, oral medication is not the treatment of choice (Nelson, 2005). Principles of DM treatment in dogs include dietary therapy, insulin replacement, exercise and prevention of multifactorial risks. Dietary therapy with high protein, high fiber and restriction of food calories by controlling amount of lipid and carbohydrate was suggested (Rucinsky et al., 2010). Instant commercial food for canine diabetes is

convenient. Insulin replacement would be considered based on veterinarian's evaluation of type of insulin, dose adjustment per day, type of DM and responsiveness. To control rapid hyperglycemia, exercise helped to increase glucose utilization (Rucinsky et al., 2010). Moreover, ovariohysterectomy of intact females and limitation of glucocorticoid administration will reduce risk factors of DM.

Although insulin administration is administered in canine DM, it is somehow difficult to stabilize blood glucose level (Catchpole et al., 2005) as long as underlying causes of DM have not yet been completely eliminated. Persistent of hyperglycemia majorly causes alteration of blood vessels via activation of protein kinase C, sorbitol accumulation and myo-inositol depletion (Kikkawa, 2000), all of which lead to other complications. They are such as atherosclerosis (Schmidt et al., 1999), diabetic ketoacidosis (DKA) (De Causmaecker et al., 2009) and peripheral neuropathy (Morgan et al., 2008). Common complications related to ocular abnormalities are such as poor corneal wound healing (Plummer et al., 2007), corneal neuropathy (Wilkie et al., 2006), keratoconjunctivitis sicca (Cullen et al., 2005), blurred vision (Sato et al., 1991) and blindness due to cataract and retinopathy (Engerman, 1989).

Diabetic cataract

In humans, incidence of diabetic cataract was 1% of diabetic population (Janghorbani et al., 2000). While in dogs, diabetic cataract developed in more than half of diabetic population (Salgado et al., 2000). Diabetic cataract would be observed from 30 to 240 days (mean 123 days), after which DM had been diagnosed (Wilkie et al., 2006). In brachycephalic dogs and Shar-pei, diabetic cataract developed in about 10 days (median value) after hyperglycemia (Davidson, 1999).

Bilateral mature diabetic cataract rapidly developed in hyperglycemic dogs with rapid lens intumescent (Ofri, 2008). Ophthalmoscopically, small vacuoles initially developed at lens equator and, afterward rapidly progressed to the entire lens in both eyes (Plummer et al., 2007). Rapid progression of DM can lead to cleft visible at the anterior suture in a Y shape (Lanckner et al., 1997), lens-induced uveitis

(Plummer et al., 2007), spontaneous lens capsule rupture (Wilkie et al., 2006) (figure 1) and sudden blindness (Fleeman and Rand, 2001).

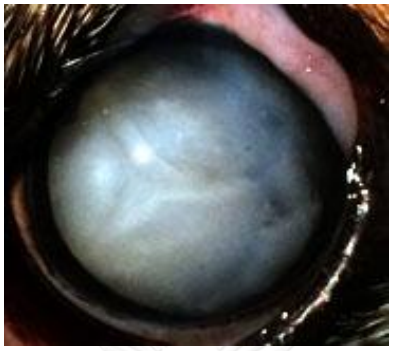

Figure 1. Characteristic of diabetic cataract in dog.
Y suture at anterior capsule is noted.

Due to the fact that there has been high level of glucose in blood vessels, concentration of glucose in aqueous humor is consequently increased. As a result, large number of glucose accumulates within the lens. Limited amount of glucose enters normal glucose metabolism (hexokinase pathway). Excessive amount of glucose in contrary enters sorbitol pathway (Davison, 2012). Instead of glucose being transformed by hexokinase via normal metabolism, saturated glucose is changed into sorbitol by aldose reductase, part of which is in turns transformed into fructose by sorbitol dehydrogenase (figure 2). Molecules of both sorbitol and fructose are considered large, so that stimulating diffusion through lens capsule. Imbibition of water within the lens induces lens intumescent (Sato et al., 1991). Aggressive amount of sorbital and fructose within the lens in associated with lens hyperosmolarity cause biological change of lens protein, leading to cataract (Davidson, 2007; Ofri, 2008).

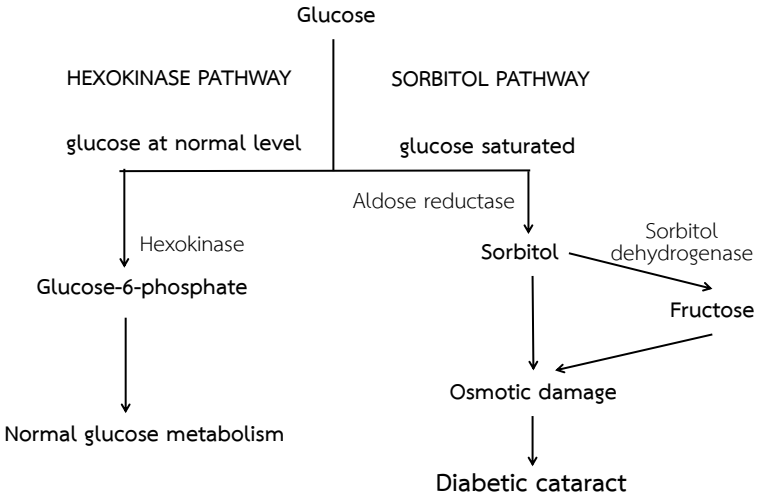

Figure 2. Diagram of glucose metabolism pathway within the lens under hyperglycemic condition.
This diagram is modified from Davison, 2007 and Ofri, 2008.

Diabetic retinopathy

Diabetic retinopathy (DR) in humans is the cause of visual impairment (Kikkawa, 2000) and 1% blindness in the population of 39 countries (Yau et al., 2012). Apparent risk of DR is long term hyperglycemia, hypertension and hyperlipidemia (Tarr et al., 2013). Pathogenesis of DR majorly involves accumulation of sorbital within retinal blood vessels (Arden and Sivaprasad, 2012), activation of protein kinase C mechanism (Kikkawa, 2000) and overproduction of oxidative stress (Tarr et al., 2013), all of which lead to a breakdown of osmotic property of retinal blood vessels. Vitreous hemorrhage usually occurs in patients with DR (Hiraiwa et al., 2003). Not only protein kinase C leads to loss of retinal vascular endothelial permeability (Chistiakov, 2011), but it also stimulates production of vascular endothelial growth factor (VEGF) (Caldwell et al., 2005), later on inducing angiogenesis. Retinal neovascularization triggered by VEGF (Arden and Sivaprasad, 2012) increases possibility of retinal hemorrhage (Hiraiwa et al., 2003) because these small, tortuous vessels (microanurysm) that lack of pericyte cells and endothelial cells are fragile

(Arden and Sivaprasad, 2012). As a consequence, retinal detachment that may be followed will cause blindness (Hiraiwa et al., 2003).

Human DR is divided into 2 types based upon characterization of retinal neovascularization (Tarr et al., 2013); non-proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR). Examination of NPDR fundus revealed macular edema, venous microanurysm and capillary nonperfusion. While in PDR (Figure 3), retinal neovascularizarion, fibrovascular proliferation, retinal and/or vitreous hemorrhage, traction retinal detachment were observed (Jacot and Vinik, 2007; Chistiakov, 2011). There are few diagnostic methods for human DR; assessment of visual acuity by Snellen test, documentation of fundus appearance by photography, evaluation of abnormal growth of retinal blood vessels by fluoresceine angiography, evaluation of macular edema by optical coherence tomography (OCT) and assessment of retinal cell function by electroretinography (ERG) (Chistiakov, 2011).

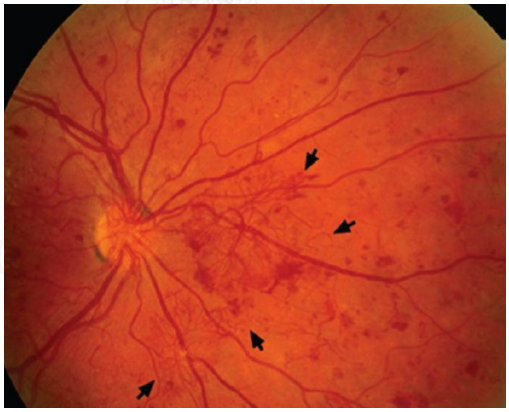

Figure 3. Fundus photograph of human proliferative diabetic retinopathy (Tarr et al., 2013).
Multiple microaneurysms, retinal hemorrhage and neovascularization (black arrows) were noted.

In dogs, incidence of DR was not as high as that of in humans (Davison, 2012). From diabetic dogs that were undergone phacoemulsification, Landry and others

(2004) reported 21% evidence of retinal hemorrhage and microaneurysm, both of which were not considered complications from phacoemulsification in dogs (Klein et al., 2011). Retinal neovascularization as well as hemorrhage, degeneration and detachment were also reported in dogs diagnosed with DM (figure 4) (Barnett, 1981). In chronic poor glycemic-controlled dogs that diabetic cataract rapidly develops, loss of vision immediately occurs due to obstruction of light by extremely opaque lens (Davidson and Nelms, 2007)

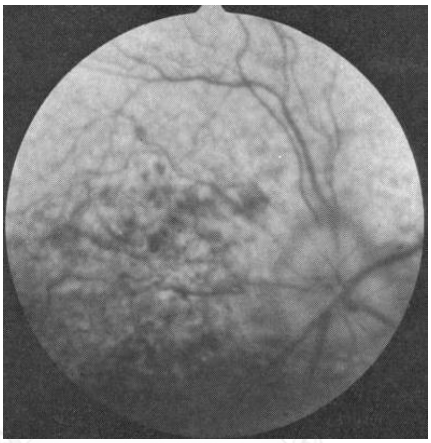

Figure 4. Fundus photograph of canine diabetic retinopathy (Barnett, 1981). Multiple retinal hemorrhage and microaneurysm were noted.

Function of the retina

Retina is composed of 10 layers (figure 5). The 10 layers from proximal to distal are retinal pigment epithelium (RPE), photoreceptor layer (PRL), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), nerve fiber layer (NFL), and inner limiting membrane (ILM).

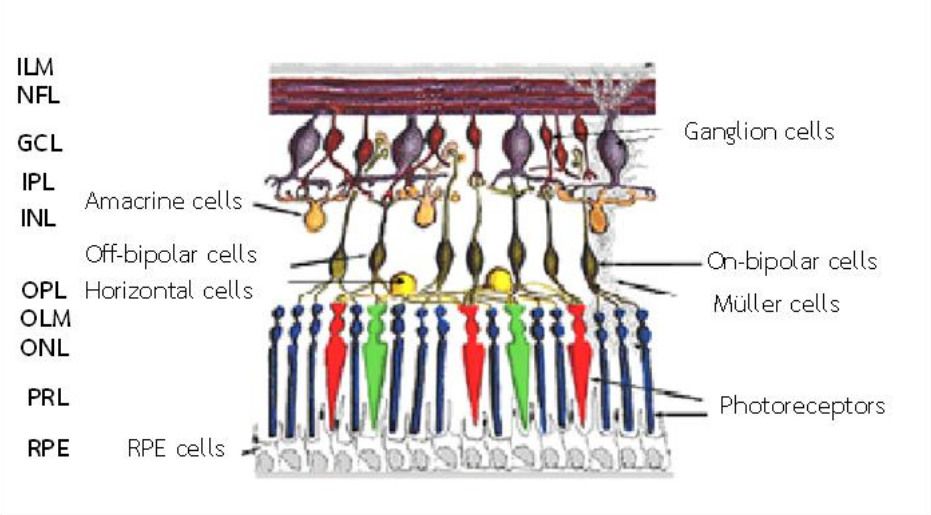

Figure 5. Schematic picture of retinal layers (Kolb, 2012).

Note: RPE= retinal pigment epithelium, PRL=photoreceptor layer, OLM=outer limiting membrane, ONL=outer nuclear layer, OPL=outer plexiform layer, INL=inner nuclear layer, IPL=inner plexiform layer, GCL=ganglion cell layer, NFL=nerve fiber layer, and ILM=inner limiting membrane.

There are 3 neuronal cells; photoreceptors (rods and cones), bipolar cells and ganglion cells. Photoreceptor segments are located in the photoreceptor cell layer. Rods are sensitive to low intensity of light like dim light environment. Cones, on the other hand, function well under bright light illumination. Moreover, cones are also responsible for higher visual acuity and color vision (Rabin, 1996). As to most diurnal mammalian species, approximately 95% of photoreceptors in dogs are rods (Koch and Rubin, 1972) whereas the rest are cones.

Retina is responsible for phototransduction. Photoreceptors contain photopigments, light-sensitive elements, essential for this mechanism. Phototransduction (figure 6) is the process by which photons are absorbed by photopigments and then converted into electrical impulses. Transmission of electrical messages as a train of action potentials occurs from photoreceptors to bipolar cells, and then to ganglion cells. Electrical impulse from axons of ganglion cells is conveyed from optic nerve to optic chiasm and optic tract before projecting

to lateral geniculate nucleus. Once impulse further transmitted via optic radiation have arrived to primary visual cortex, it is transformed into visual image (Ofri, 2008).

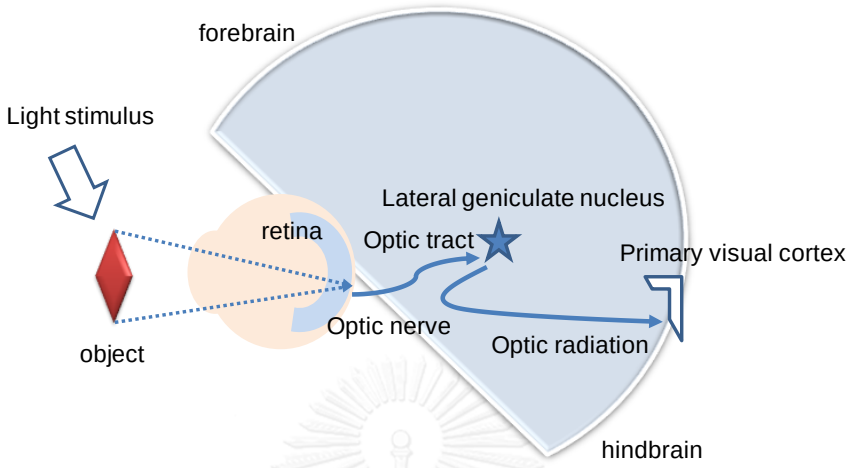

Figure 6. Schematic diagram of phototransduction cascade.

Electroretinography

Electroretinography (ERG) is the method to quantitatively measure retinal function by light stimulation to the retina. Summation of electrical activities throughout the entire retina can be recorded via the corneal surface. Full-field Flash ERG is one of efficient techniques to examine retinal function using homogeneous light stimulus (Narfström et al., 2002). Eyes receive indirect light reflection from a spherical (ganzfeld) bowl of which the inferior surface is diffusely coated with reflective spectrally non-selective material. Stimulation of retina with various light intensities; flash intensity-series, not only can provide more information of retina capability to responding to increasing light intensity (Ekesten et al., 2013), but also retinal sensitivity to minimal light stimulus.

ERG is often used to diagnose several canine retinal diseases for examples: progressive retinal atrophy (PRA), sudden acquired retinal degeneration (SARDs), assessment retinal function before cataract surgery, differentiation of blindness

caused by retinal or brain abnormality and retinal function affected from some medicine or toxins (Ofri, 2008; Ekesten et al., 2013).

Many factors, such as biological factors (age, breed, and gender), recording techniques, type of light stimulation or anesthetic protocol, can affect ERG recording (Komáromy et al., 2002). To ensure that ERG interpretation is accurate while ERG procedure is producible, it should be performed with uniformity worldwide. Global standardization of ERG for humans had been set by the International Society for Clinical Electrophysiology of Vision (ISCEV) (Marmor et al., 2009). Flash ERG according to condition of adaptation and flash stimulus (candelas second per square meter; cds/m^2) recommended by ISCEV consists of minimum 5 responses to clinically assess retinal function (figure 7).

Flash ERG protocol recommended by ISCEV are described: 1. Dark-adapted 0.01 ERG, 2. Dark-adapted 3.0 ERG, 3. Dark-adapted 3.0 oscillatory potentials, 4. Light-adapted 3.0 ERG and 5. Light-adapted 3.0 Flicker ERG.

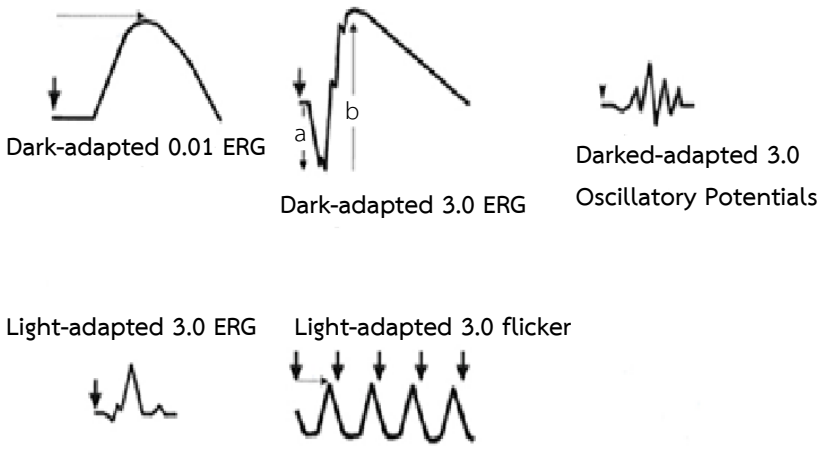

Figure 7. Standard flash ERG responses set by the International Society for Clinical Electrophysiology of Vision (Marmor et al., 2009).

To achieve international standardization like in humans, canine full-field, flash ERG protocol had been set by the European College of Veterinary Ophthalmologists (ECVO) (Ekesten et al., 2013). There are 3 major sections described; short description of the basis for the test of rod and cone function in the dog, some technical aspects

of the recording, such as equipment and patient preparation and a summary of the recommended protocol and the optional add-ons. Most of the protocol, including 5 basic flash stimulations, is comparable to that of in humans; however, few differences were noted. Light intensity of 0.01 or 0.02 cds/m^2 can be selected for dark adapted rod response examination. While the test of OPs is optional in dogs, general anesthesia is necessary to reduce electrical artifacts due to involuntary oculomuscular movement.

For clinical ERG waveform, the first occurring negative potential is a-wave that originates from hyperpolarization of photoreceptors. The first occurring positive potential that immediately follows a-wave is b-wave. It originates from depolarization of bipolar cells in combination with Müller cells and represents function of the inner retina. Function of ERG is reported by amplitude (microvolt; μV) and implicit time (millisecond; ms) of the waveform (Narfström et al., 2002; Ofri, 2008; Ekesten et al., 2013). Four wavelets superimposed on the ascending and the top of the b-wave are oscillatory potentials (OPs). Origin of OPs is still unclear, but it is related to transmissible cooperation between bipolar cells, amacrine cells and gial cells (Asi and Perlman, 1992; Shirao and Kawasaki, 1998). Flicker is the frequency of flash stimulation that depends on function of photoreceptors under the prevailing levels of illumination (Ofri, 2008).

Electroretinography in patients with DR

Alteration of retinal vessels can only be observed in PDR patients via fundus examination (Chistiakov, 2011), but yet change of retinal function cannot be assessed. ERG has become an alternative test to investigate retinal function in DR patients. Due to the fact that ERG is a sensitive method, it could be applied to investigate retinal change in early stage of DR not associated with alteration of retinal vessels (Parisi et al., 1997; Shirao and Kawasaki, 1998). Kim and others (1997) detected change of human OP wavelets; reduction of amplitude and delay peak latency. These alterations may be related to retinal ischemia. In PDR patients, it was recommended by Hirose (1977) to investigate the ratio of b- to a-wave amplitude

(b/a ratio). Patients with b/a ratio above 1.0 had a better chance of improved vision after surgery, especially with correction of vitreous hemorrhage by vitrectomy. When b/a ratio of the combined cone-rod response is below 1.0, it is an indication of central artery occlusion and severe central vein occlusion (Koh et al., 2001).

ERG responses in diabetic mouse fed with galactose diet showed a reduction in a- and b-wave amplitudes in association with prolonged OP implicit time (Robinson et al., 2012). DM-induced rats by streptozotocin had a decrease in photoreceptor response within 12 weeks (Phipps et al., 2006) while alteration of ERG responses in DM-induced rat by the same drug were observed prior to a detection of changes in retinal vascular structure (Kohzaki et al., 2008). In diabetic dogs, reduction of b-wave amplitude together with prolong b-wave implicit time were reported (Safatle et al., 2010). Further, prolonged b-wave implicit time was statistically significant at 30 Hz.

Due to the fact that diabetic dogs usually develop severe bilateral cataracts, not only vision is impaired, fundus cannot be investigated by routine examination. Vision following cataract surgery is therefore doubtful. Pathological changes of the retina in humans with DR are well recognized while documentation of retinal function in diabetic dogs is limited. ERG is a sensible procedure to detect abnormalities of retinal cells in patients without visible retinopathy (Tyrberg et al., 2011). We herein report characterization of a full-field intensity-series ERG in dogs with diabetic cataract.

Chapter 3
Materials and methods

Animals

Fifty dogs from Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University were included in this study. All dogs had cataract, of which the stage had impaired vision during the day. The detail of fundus could not be observed. Control group consisted of twenty five non-diabetic dogs (n=25) from Ophthalmology Clinic who had fasting blood glucose level within normal limit (70-90 mg/dl). They had no previous history of diabetes mellitus.

Twenty five diabetic dogs (n=25) from Diabetic Clinic were in experimental group. Fasting blood glucose was assessed using glucometer (glucometer; Accu-Chek® Performa, Frontier medical, Auckland, New Zealand). Diabetes mellitus were diagnosed when fasting blood glucose level was greater than 250 mg/dl (Holland, 2010), at which glucosuria and ketonuria were involved and confirmed by urinalysis (Davison, 2012). In addition, their serum fructosamine was greater than 400 µmol/l (Davison, 2012). Polyuria and polydipsia were apparent. All dogs were treated with DM immediately after diagnosis.

To select candidates of this study, all dogs were undergone thorough ophthalmic examinations in both light and dark conditions. Other ocular abnormalities must not be involved. Ophthalmic examinations included basic neuro-ophthalmic reflexes (menace response, dazzle reflex, pupillary light response, and blink reflex), quantity of tear production via Schirmer Tear Test I, examinations of ocular adnexa, fluorescein staining test, intraocular pressure measurement using rebound tonometer (TonoVet® ; Icare Finland, Helsinki, Finland), condition of cataractous lens through dilated pupil and investigation of retinal detachment using ocular ultrasonography (Ocular ultrasonographic machine, Ultrascan® imaging system, Alcon Laboratories, Inc, Hünenberg, Switzerland).

All dogs were allowed to participate in this study according to Chulalongkorn University Animal Care and Use Committee, (No. 1431030). The owner had been informed detail of this study and requested to sign a consent form.

Animal preparation

Routine blood examinations were performed one week prior to ERG testing. Blood chemistry profiles included serum glutamic-pyruvic transminase (SGPT; IU/L), alkaline phosphatase (ALP; IU/L), creatinine (mg/dl), BUN (blood urea nitrogen; mg/dl). Complete blood count profiles were red blood cell count (RBC; cells/μ), red blood cell morphology, white blood cell count (WBC; cells/μ), white blood cell differentiation and platelet count (cells/μ). Investigation of blood parasite manifestation was also performed Thoracic radiography and echo cardiography was required in dogs older than 8 years of age. In diabetic dogs, fasting blood glucose level had to be maintained at the level less than 250 mg/dl, while serum fructosamine level was maintained below 400 μmol/l.

Three days prior to ERG testing, 1.0% atropine sulfate (1.0% Isopto atropine®; Alcon Laboratories, Thailand) was topically administered twice daily to achieve fully pupil dilation. On the ERG tested date, topical administration of 10% tropicamide (Mydriacyl; Alcon Laboratories, Thailand) interchangeable with 10% phenylephrine (Phenylephrine HCL; Biolab, Thailand) was applied every 10 minutes for one hour to ensure permanent pupil dilation until the end of the recording. Retinal detachment was ruled out by B-scan ocular ultrasonography.

All dogs were scheduled for ERG testing in the morning to avoid alteration of blood glucose level. Food and drink had been withheld for 6 hours before the time of general anesthesia. Following re-examination of blood glucose, a quarter to half dose of insulin administration was considered if fasting blood glucose level in diabetic dogs was above 78 mg/dl. This protocol aiming to maintain glucose level during anesthesia in diabetic dogs was recommended by Duddale (2010) and Holland (2010) to avoid hypoglycemia leading to brain edema and seizure.

Anesthetic procedure

Each dog was pre-medicated by 0.3 mg/kg morphine sulfate (Morphine sulfate; Food and Drug Administration, Thailand) combined with 0.01-0.03 mg/kg acepromazine maleate (Combistress®; Anitech Total Solution, Thailand) intramuscularly. Dogs were induced by 1% of 3-6 mg/kg propofol (Lipuro®; B. Braun, Germany), followed by a maintenance with inhaling isoflurane (Attane™; Piramal Critical Care, Inc, U.S.A.) delivered in oxygen at 200 milliliter per kilogram (ml/kg) flow rate. Lactated ringer's solution (LRI; Thai nakornpattana, Thailand) was intravenously given during anesthesia, and then was maintained at 10 milliliter per kilograms of body weight per hour in the first hour (ml/kg/h) and 5 ml/kg/h in the second hour.

Anesthetic parameters; mental status, mucous membrane coloration, capillary refilling time, hydrating status, body temperature, heart rate, respiratory rate, heart sound and lung sound auscultation, were monitored and recorded every 10 minutes throughout anesthesia. In order to maintain blood glucose in diabetic dogs at level between 150 and 250 mg/dl (Bednarski et al., 2011), level of blood glucose was monitored every 30 minutes during general anesthesia. When blood glucose level was above 250 mg/dl, a quarter to normal dose of insulin was administered via subcutaneous route. If blood glucose level was below 150 mg/dl, Lactated ringer's solution was replaced by 5% dextrose in water (D-5-W; Thai Otsuka Pharmaceutical, Thailand) until glucose level returned to normal range.

Monitoring of vital signs was continued until dogs fully recovered. Blood glucose level in diabetic dogs was repeatedly checked to ensue at level below 250 mg/dl.

Electroretinographic procedure

Electroretinographic machine: ERG responses were recorded using electrophysiology unit (RetiPort 32 Version 4.3.8; ROLAND CONSULT, Brandenburg an der Havel, Germany). The unit consists of ganzfeld bowl with light stimulator unit

(figure 10A), amplifier and computer analyzing system, all of which were connected to each other (figure 10B).

Placement of recording electrode: Three electrodes were required to complete electrical circuit for recording; recording, reference and ground electrode. Globe was positioned in a primary gaze with stay sutures of 4-0 nylon (ETHILON*; Polyamide; Johnson and Johnson Intl, Belgium). Corneal contact lens electrode (JET lens; ERG-jet®; Fibrinal SA, Switzerland) (figure 8A) was used as a recording electrode. It was placed on the cornea with lubrication of 0.2% methylcellulose (Visidic®; Bausch & Lomb U.K Limited, Germany) to enhance electrical conductivity and moist the cornea. Silver needle (Needle Electrode Connection; ROLAND CONSULT, Brandenburg an der Havel, Germany) (figure 8B) was used as a reference electrode by placing subcutaneously at the temporal region of testing eye. As a ground electrode (figure 8C), silver needle was subcutaneously placed at the external occipital protuberance area. All electrodes were directed to the amplifier, which was then connected to ERG computerized unit (figure 9).

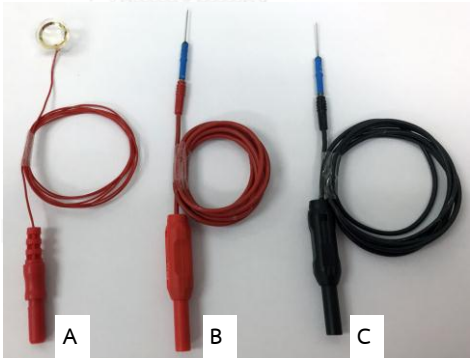

Figure 8. Electrodes required for ERG recording.
(A) JET contact lens electrode; (B) needle reference electrode; and (C) needle ground electrode.

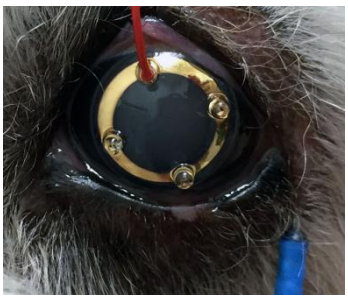

Figure 9. Placement of JET lens and needle electrode for canine ERG recording.

Figure 10. Electrophysiological units.
(A) Ganzfeld bowl connected to the amplifier; (B) ERG computerized unit.

Animal positioning for ERG testing: Dog was positioned in sternal recumbency on a wooden wheeled sleigh. His head was positioned on a small wooden box slightly raised above the level of a sleigh (figure 11). Once all electrodes had been connected, the dog was moved toward the ganzfeld bowl to ensure that both eyes were behind the opening of the bowl.

Figure 11. Animal positioning for ERG testing.

Electroretinographic testing protocol: Intensity-series full field ERG was performed in this study. The time base was set to record at 0 msec. Inter-stimulus interval of each flash intensity was set, while ERG responses of each individual flash intensity were averaged.

Scotopic intensity-series electroretinography

To stimulate rod function, eyes were dark-adapted for 30 minutes by being covered with occlusive bandage. ERG responses from a series of seven white flash stimuli (0.003, 0.0095, 0.03, 0.095, 0.3, 0.95 cds/m^2 and 3.0 cds/m^2) were recorded respectively. Oscillatory potentials were electronically isolated from scotopic stimulus of 3.0 cds/m^2. Inter-stimulus intervals were increased from 1 second at low intensities to 360 seconds at the highest intensity. Stimulus intensities included in this study were set in accordance with the ISCEV standard (Marmor et al., 2009). ERG responses were computerized and stored for further analysis.

Photopic intensity-series electroretinography

To stimulate cone function, dogs were light-adapted to a rod saturating white background of 30 cd/m^2 for 10 minutes after scotopic ERG had been recorded. Photopic responses were recorded from a series of 2 white flashes (3.0 and 9.5 cds/m^2), superimposed on the same background white light. Flicker ERGs were recorded thereafter using white flash stimulus of 3.0 cds/m^2 at 30 Hz, and 10 tracings averaged. Stimulus intensities included in this study were also recommended by

ISCEV standard (Marmor et al., 2009). ERG responses were computerized and stored for further analysis.

Data analysis

The a- and b-wave amplitude and implicit time: The a- and b-wave amplitude (microvolt; μV) and implicit time (millisecond; msec) were measured for each averaged response. Amplitude of the a-wave was measured from the onset of light stimulus to the trough of the first negative wave; while amplitude of the b-wave was measured from the trough of the first negative wave to the peak of the first positive wave (Aroch et al., 2008).

A-wave implicit time was time measured from the onset of the light stimulus to the time when the maximal a-wave trough occurred. B-wave implicit time was time measured from the onset of the light stimulus to the time when the peak b-wave was present (figure 12 and figure 13). Mean (± SE) of scotopic and photopic ERG amplitudes and implicit times were calculated and plotted as a function of light stimulus.

The b/a ratio at light intensity of 3.0 cds/m^2 was achieved by a division of b-wave amplitude by a-wave amplitude.

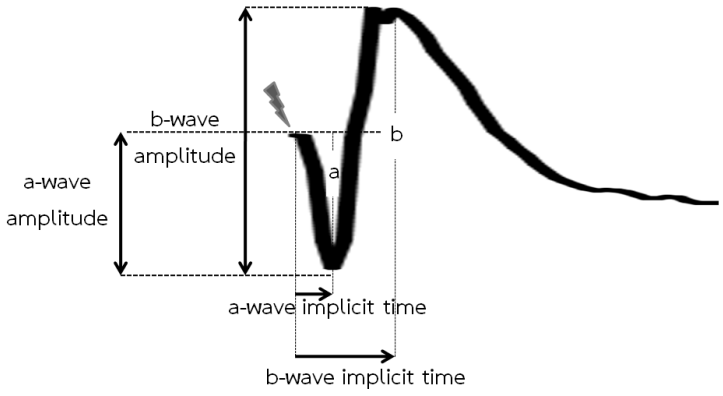

Figure 12. Scotopic ERG waveform.
Measurements of amplitude and implicit time of a- and b-wave are demonstrated. Note that striking sign indicates onset of light stimulus.

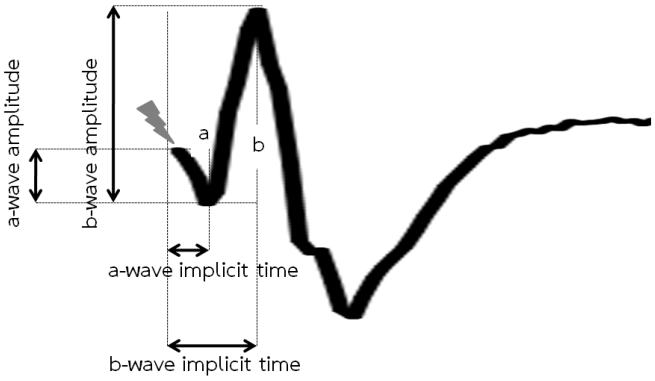

Figure 13. Photopic ERG waveform.
Measurements of amplitude and implicit time of a- and b-wave are demonstrated. Note that striking sign indicates onset of light stimulus.

OP_2 amplitude was measured from the tough of OP_1 to the preceding positive peak, while implicit time was measured from the time at which light was stimulated to the time when peak of OP_2 was present (figure 14) (Shirao and Kawasaki, 1998; Tzekov and Arden, 1999).

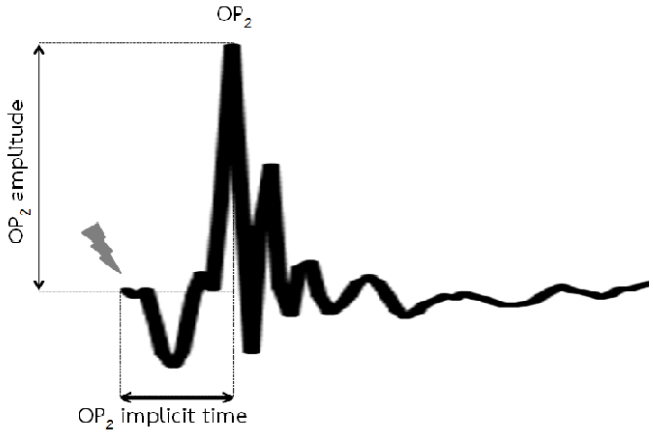

Figure 14. Oscillatory potential.
Measurements of amplitude and implicit time of OP_2 are demonstrated. Note that striking sign indicates onset of light stimulus.

Ten flicker responses were analyzed. Amplitude and implicit time were measured for an entire recording period. Amplitude was measured from trough to peak of each wave while implicit time was measured from the time of light onset to each peak of response (figure 15) (Safatle et al., 2010).

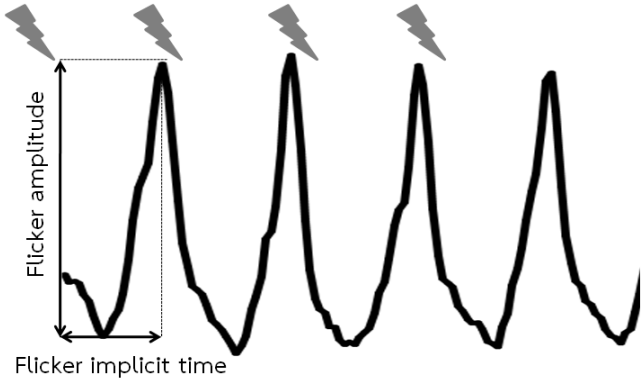

Figure 15. Flicker response.
Measurements of amplitude and implicit time are demonstrated. Note that striking sign indicates onset of light stimulus.

Statistical analysis

Mean difference of ERG amplitude and implicit time were statistically analyzed via independent t-test with a significant level at $p \leq 0.05$ using SPSS program (IBM SPSS statistics Version 19.0, SPSS Inc.).

Chapter 4

Results

Age of diabetic dogs was ranged from 5 to 15 years with the mean age of 9.52 years, whereas it was from 5 to 17 years with the mean age of 9.68 years in non-diabetic group. Number of males and females was equal (25 dogs of each gender). Dog breeds were described in table 1. Mean duration that diabetes mellitus had been diagnosed was 17 months; ranged from 3 to 39 months. Cataract appeared almost at mature stage. All dogs had impaired vision due to cataract condition.

Table 1. Number of dogs according to breed and diabetic status.

Breeds	Total number of dogs	Number of diabetic dogs	Number of non-diabetic dogs
Mixed breed	19	8	11
Poodle	12	6	6
Miniature pincher	4	2	2
Shih tzu	3	2	1
Chihuahua	2	1	1
Yorkshire terrier	2	1	1
Siberian husky	2	1	1
Pug	2	1	1
Pomeranian	2	1	1
Dachshund	1	1	-
Thai Bangkaew	1	1	-
Total	50	25	25

Scotopic ERG responses

Dogs had increasing ERG responses with intensity-series stimuli. Two different patterns of ERG waveform were observed in diabetic group. First, it was a character of ERG waveforms that were comparable with those of normal (figure 16), but lower in responses. In diabetic dogs, scotopic responses were present according to all light stimuli though proportional reduction of responses was observed. This comparable characteristic of ERG waveform was noted in 37 diabetic eyes from 19 diabetic dogs.

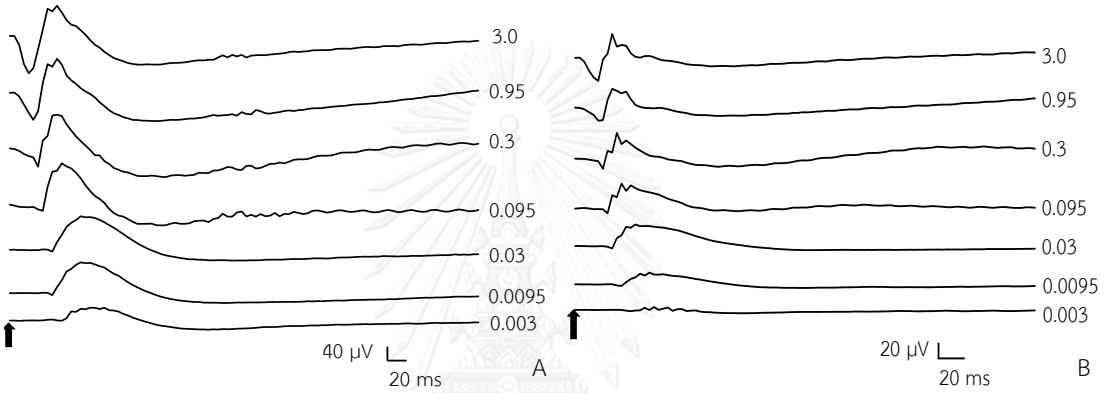

Figure 16. Representative scotopic ERG recordings from diabetic (A) and non-diabetic dogs (B).

Light intensities (0.003, 0.0095, 0.03, 0.095, 0.3, 0.95 and 30 cds/m^2) are indicated in the figure. Vertical and horizontal size bars indicates amplitude in microvolt and implicit time in millisecond, respectively. Note that black arrow is the onset of light stimulus.

The other characteristic of ERG waveform in diabetic dogs was defined as a 'negative' ERG response. Presence of small b-wave was not proportional as compared to that of highly negative a-wave (figure 17). This pattern of ERG response was noted in 11 eyes from 6 dogs (mixed breed; n=2, Miniature pincher; n=2, Miniature poodle; n=1 and Chihuahua; n=1). Although scotopic responses in diabetic group were present according to all light stimuli, reduction of responses was remarkable.

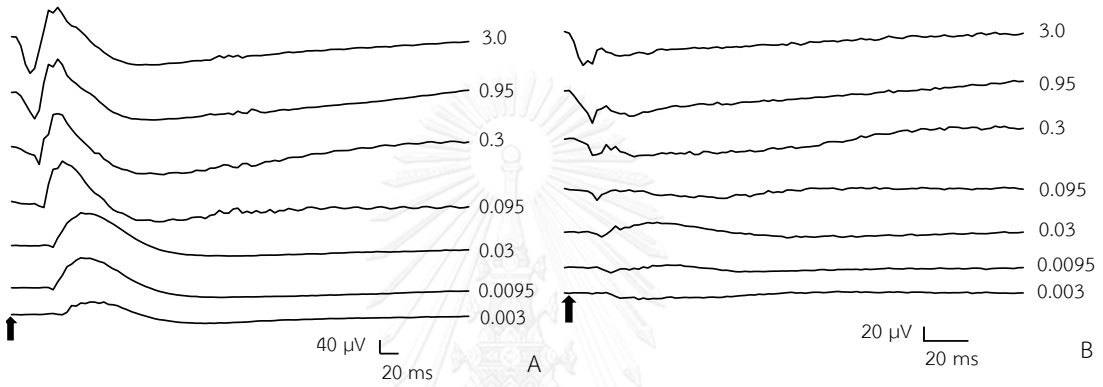

Figure 17. Representative scotopic ERG recordings from diabetic (A) and non-diabetic dogs (B).

The diabetic dog demonstrated 'negative' ERG waveform that b wave does not exceed a wave. This characteristic of ERG response was notable from light stimulus 0.0095 cds/m^2 toward the highest light intensity. Light intensities (0.003, 0.0095, 0.03, 0.095, 0.3, 0.95 and 30 cds/m^2) are indicated in the figure. Vertical and horizontal size bars indicates amplitude in microvolt and implicit time in millisecond, respectively. Note that black arrow is the onset of light stimulus.

Mean ERG amplitudes of diabetic eyes were significantly low compared to that of non-diabetic eyes at all light stimuli (figure 18 and figure 19). In both groups, a-wave was first observed at 0.095 cds/m^2 while b-wave began since 0.003 cds/m^2. In diabetic eyes, minimal mean a- and b-wave amplitudes were 5.6 ± 0.6 µV and 13.8 2.3 µV, respectively. According to intensity-series stimuli, mean a- and b-wave

amplitudes increased and reached maximal amplitudes of 52.2 ± 3.72 μV and 96 ± 9.16 μV, respectively.

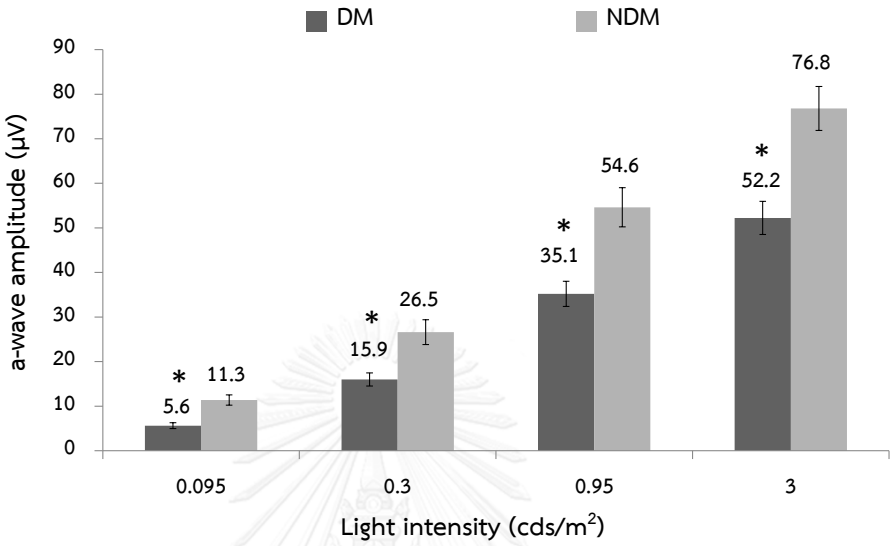

Figure 18. Mean (± SE) scotopic a-wave amplitudes of diabetic (DM) and non-diabetic (NDM) dogs.

Note that star sign indicates statistical difference at $p \leq 0.05$.

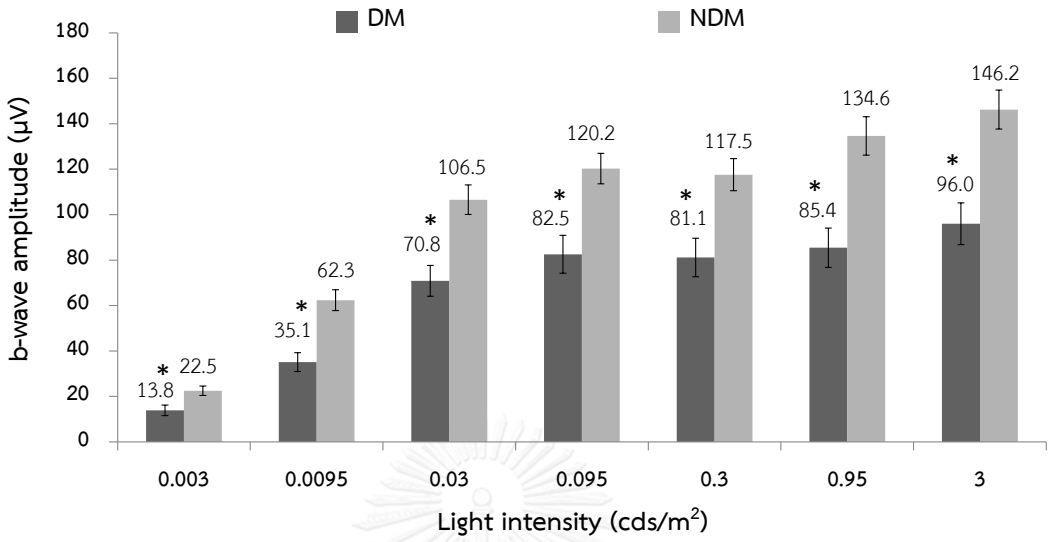

Figure 19. Mean (± SE) scotopic b-wave amplitudes of diabetic (DM) and non-diabetic (NDM) dogs.

Note that star sign indicates statistical difference at $p \leq 0.05$.

Traditionally, implicit times were inversely related to increasing light intensities. Mean ERG a- and b-wave implicit times of diabetic eyes were long compared to those of non-diabetic eyes (figure 20 and figure 21). No statistical difference was observed between the two groups of experiment. At the brightest intensity, mean a-wave implicit time was 18.5 ± 0.38 and 18 ± 0.39 ms in diabetic and non-diabetic eyes, respectively. Meanwhile mean b-wave implicit time was 39.6 ± 1.25 and 39.3 ± 1.05 ms in diabetic and non-diabetic eyes respectively.

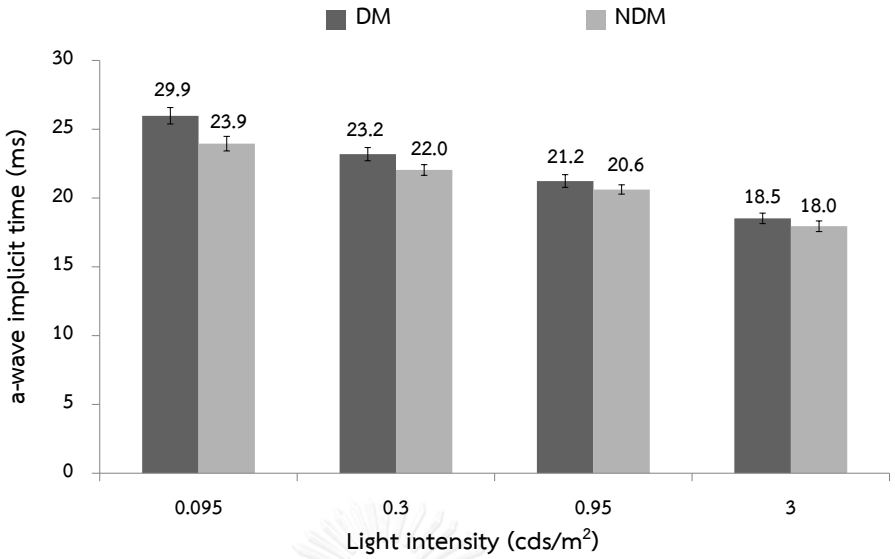

Figure 20. Mean (± SE) scotopic a-wave implicit time of diabetic (DM) and non-diabetic (NDM) dogs.

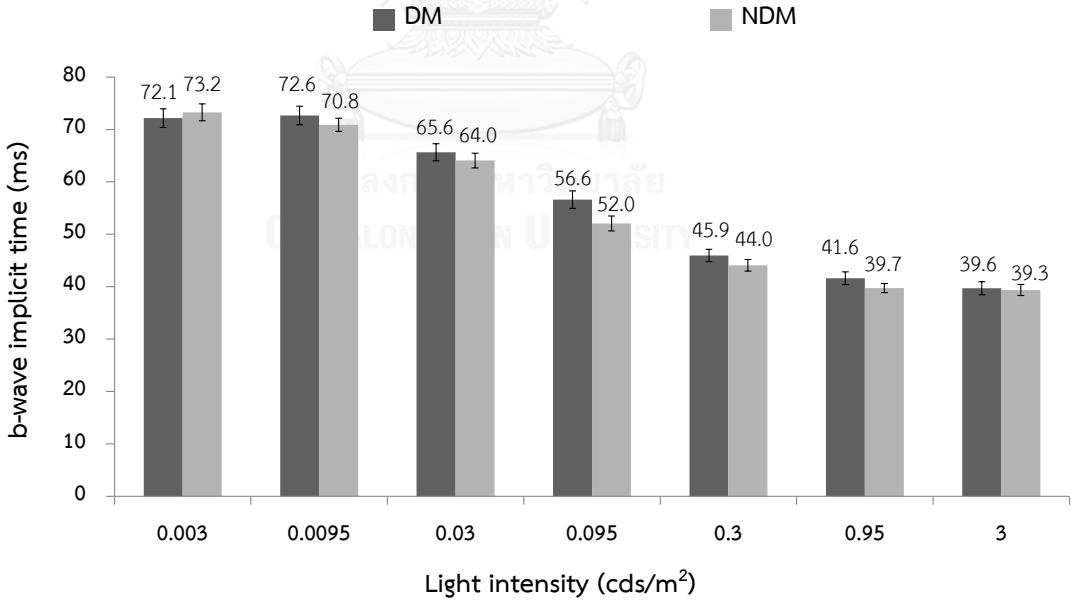

Figure 21. Mean (± SE) scotopic b-wave implicit time of diabetic (DM) and non-diabetic (NDM) dogs.

Note that star sign indicates statistical difference at $p \leq 0.05$.

The mean b/a ratio in diabetic eyes was 1.77 while it was 2.01 in non-diabetic eyes. Slight low b/a ratio was observed in diabetic group. However, statistically significant difference was not indicated (figure 22).

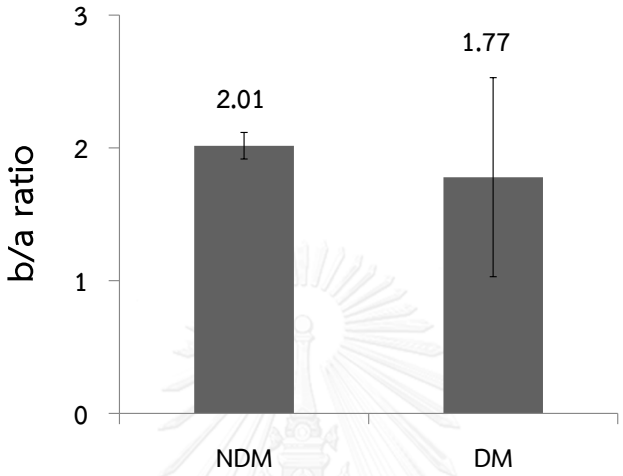

Figure 22. Mean (± SE) scotopic b/a ratio of diabetic (DM) and non-diabetic (NDM) dogs.

Decreased OP amplitude and delayed OP implicit time were observed in all OP wavelets in diabetic eye (figure 23). While mean OP amplitude in diabetic eyes was statistically significantly lower than that of non-diabetic eyes, mean implicit time of diabetic eyes was statistically significantly delayed compared to that of non-diabetic eyes (figure 24). Within a group of diabetic eyes, the group that had negative ERG character showed lower OP response compared to the other.

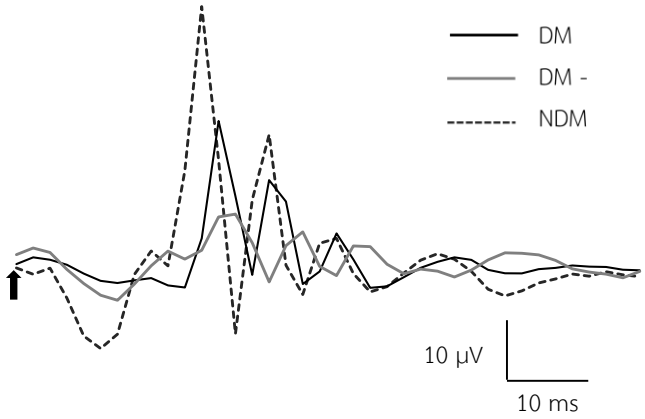

Figure 23. Representative oscillatory potential from diabetic and non-diabetic dogs. Vertical and horizontal size bars indicates amplitude in microvolt and implicit time in millisecond, respectively. Note that black arrow is the onset of light stimulus; DM=diabetic; NDM=non-diabetic.

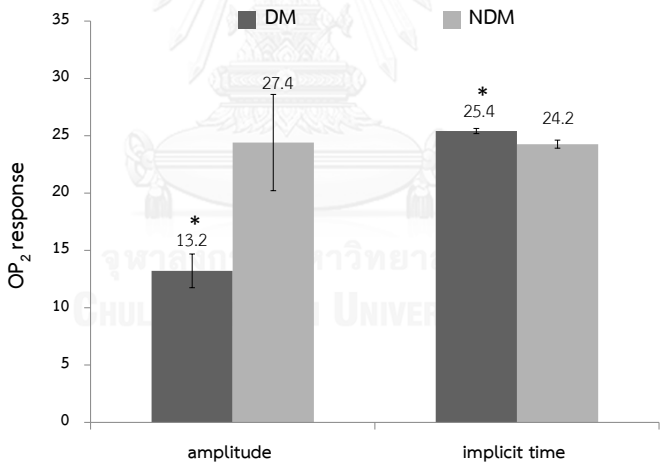

Figure 24. Mean (± SE) oscillatory potential amplitude (μV) and implicit time (ms) of diabetic (DM) and non-diabetic (NDM) dogs.
Note that star sign indicates statistical difference at $p \leq 0.05$.

Photopic ERG responses

As to scotopic ERG response, two different patterns of photopic ERG waveform were as well observed in diabetic group. In the group that character of ERG waveforms was comparable with those of controls (figure 25), proportional reduction of a- and b-wave photopic responses were noticed. Brighter light stimulus contributed bigger ERG response in diabetic eye though it was lower than that of non-diabetic eye.

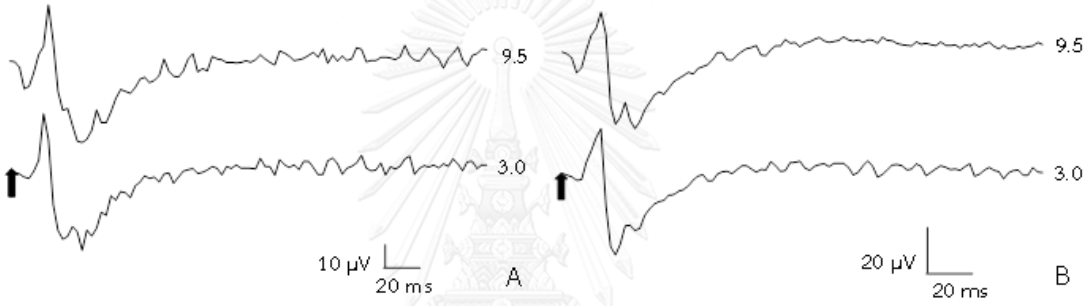

Figure 25. Representative photopic ERG recordings from diabetic (A) and non-diabetic dog (B). Light intensities (3.0 and 9.5 cds/m2) are indicated in the figure.
Vertical and horizontal size bars indicates amplitude in microvolt and implicit time in millisecond, respectively. Note that black arrow is the onset of light stimulus.

In the group with negative ERG response, unusual photopic a-wave was also observed. As light stimulus increased, negative a-wave was more prominent while b-wave markedly reduced (figure 26).

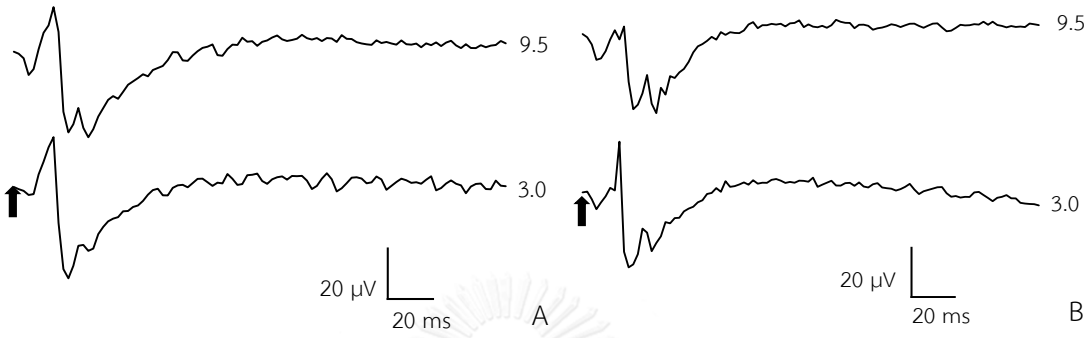

Figure 26. Representative photopic ERG recordings from diabetic (A) and non-diabetic dog (B).

This diabetic dog demonstrated negative waveform that b wave does not exceed a wave at 9.5 cds/m^2. Light intensities (3.0 and 9.5 cds/m^2) are indicated in the figure. Vertical and horizontal size bars indicates amplitude in microvolt and implicit time in millisecond, respectively. Note that black arrow is the onset of light stimulus.

Mean photopic ERG amplitudes in diabetic group were statistically significantly lower than those in non-diabetic group (figure 27). Maximal mean photopic a-wave amplitude was 7.3 ± 0.66 and 12.5 ± 1.61 μV in diabetic and non-diabetic group, respectively. Statistically significant decrease in maximal mean photopic b-wave was also apparent in diabetic eyes (14.7 ± 1.17 μV) as compared to in non-diabetic eyes (23.2 ± 1.85 μV) (figure 28).

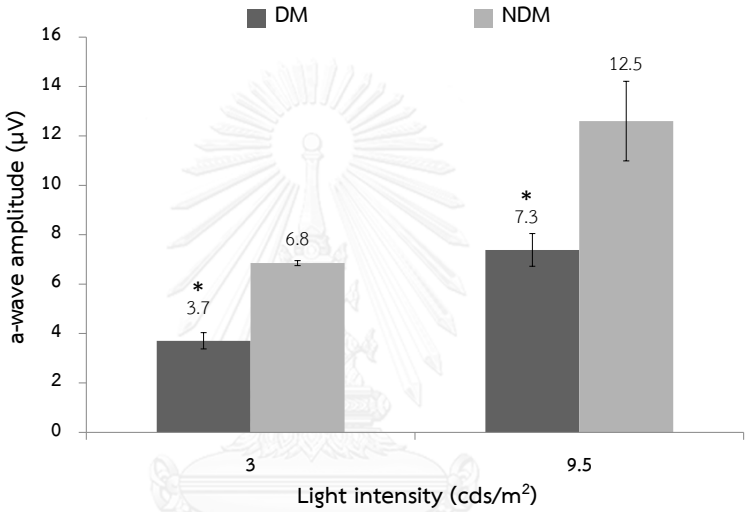

Figure 27. Mean (± SE) photopic a-wave amplitudes of diabetic (DM) and non-diabetic (NDM) dogs.

Note that star sign indicates statistical difference at $p \leq 0.05$.

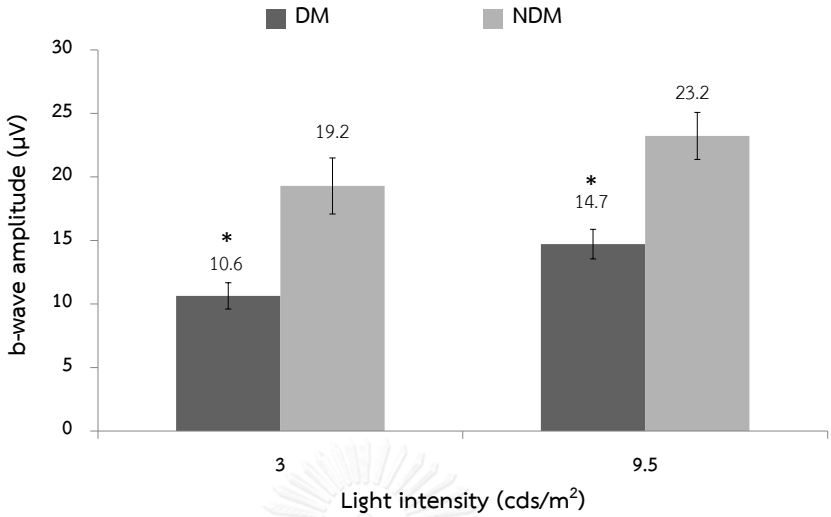

Figure 28. Mean (± SE) photopic b-wave amplitudes of diabetic (DM) and non-diabetic (NDM) dogs.
Note that star sign indicates statistical difference at $p \leq 0.05$.

Mean photopic a-wave implicit time tended to slight delay in diabetic group, compared to that of non-diabetic group (figure 29). In contrary, mean photopic b-wave implicit time in diabetic group was more rapid (figure 30). However, significant difference was not found between the two groups of experiment.

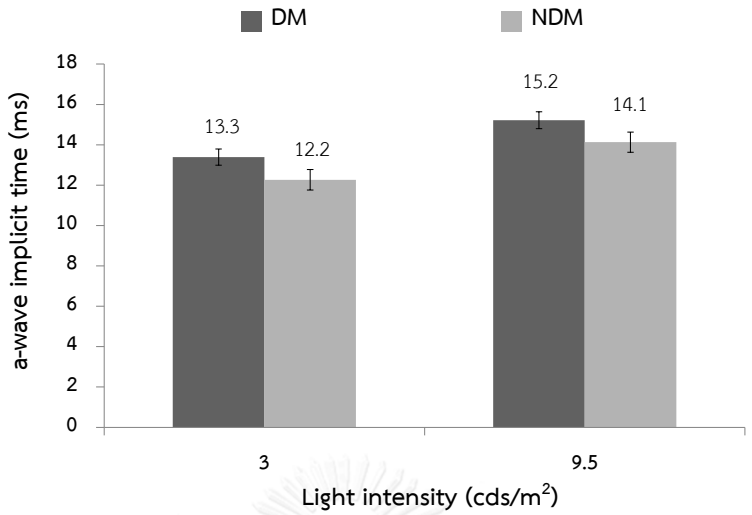

Figure 29. Mean (± SE) photopic a-wave implicit times of diabetic (DM) and non-diabetic (NDM) dogs.

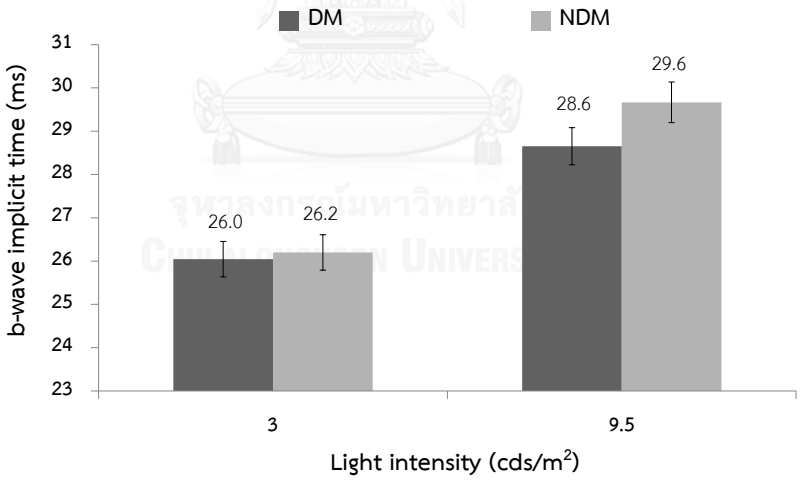

Figure 30. Mean (± SE) photopic b-wave implicit times of diabetic (DM) and non-diabetic (NDM) dogs.

In diabetic group presenting analogous characteristic of ERG response to normal, similar flicker waveform was noticed. Reduction of flicker amplitude was observed in diabetic eyes presenting negative ERG character (figure 31). Slight delay

of flicker implicit time was observed in diabetic eye; more prolonged implicit time was seen in the eye with negative ERG response.

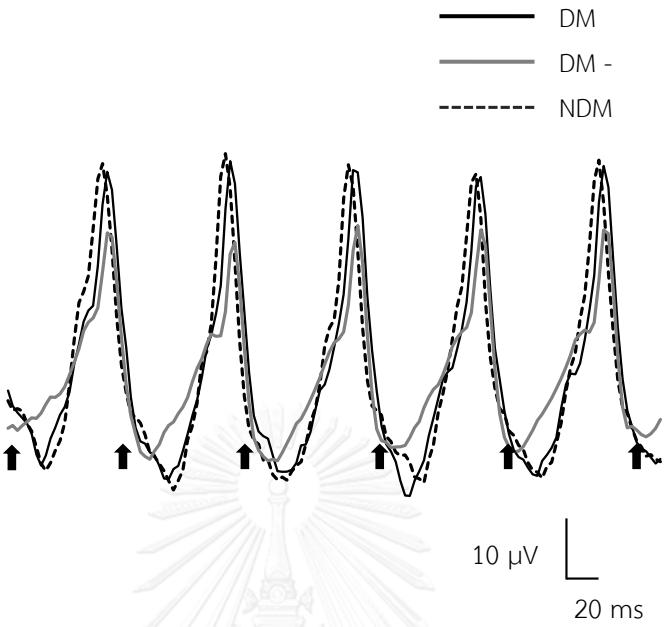

Figure 31. Representative flicker response from diabetic and non-diabetic dogs. Vertical and horizontal size bars indicates amplitude in microvolt and implicit time in millisecond, respectively. Note that black arrow is the onset of light stimulus throughout the record; DM=diabetic; NDM=non-diabetic.

Mean amplitude of flicker response was 28 ± 2.2 µV in diabetic eyes where as it was 40 ± 3.2 µV in normal. Statistical difference of flicker amplitude between two groups of experiment was indicated (figure 32). Mean implicit time on the other hand, was not statistically different. It was 26.7 ±2.0 and 27.6 ±0.4 ms in diabetic and non-diabetic group, respectively.

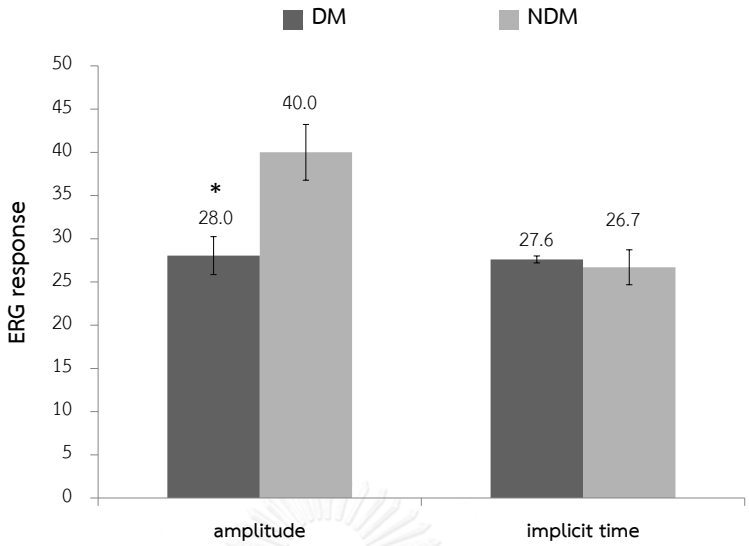

Figure 32. Mean (± SE) flicker amplitude (μV) and implicit time (ms) of diabetic (DM) and non-diabetic (NDM) dogs.

Note that star sign indicates statistical difference at $p \leq 0.05$.

Chapter 5
Discussion and Conclusion

With the use of full-field intensity series white flash ERG, we have in this study revealed several changes in the retina of diabetic dogs. Significant reduction of the ERG in both dark and light conditions is parallel to the study in diabetic dogs, demonstrated delayed implicit time (Safatle et al., 2010), diabetic patients with reduced OP responses (Parisi et al., 1997; Movasat et al., 2008; Tyrberg et al., 2011; Nasralah et al., 2013) and experimental mice with reduction of ERG amplitudes (Phipps et al., 2006). Comparison of the ERG recording between normal dogs and experiments is highly controlled to minimize confounding variables (Ekesten et al., 2013).

Photoreceptors are influenced by hyperglycemia. Histologically, outer segments are shortened resulting in loss of attachment between segments and retinal pigment epithelium (Breton et al., 1995). Besides, glucose fluctuation can lead to ionic flux of retinal pigment epithelium (Schneck et al., 2000). Failure of blood supply to photoreceptors, via choriocapillaries confirmed by MRI (Berkowitz et al., 2004), occurs and causes photoreceptor apoptosis afterward (Osborne et al., 2004). As a consequence of photoreceptor cell death, mean a-wave amplitudes are significantly low. According to limited post-receptoral electrical impulse and reduced neuronal transmission from photoreceptors to bipolar cells (Greenstein et al., 1992; Satoh et al., 1993), reduction of the mean b-wave amplitudes are therefore detected in our study. Reduction of a-wave sensitivity in human DR as found in diabetic rats induced by streptozotocin was correlated to a decrease in number of G-protein, which is part of phototransduction cascade (Kowluru et al., 1992). Although a-wave implicit time is not statistically reduced, delay in a-wave response indicates that photoreceptor sensitivity in dogs with DM is not well preserved.

Na^+/K^+-ATPase is majorly required for photoreceptor inner segments function (Wetzel et al., 1999). Reduction of ATPase activity that was reported in diabetic rats

induced by streptozotocin (Phipps et al., 2006) may be accounted for substantial deficit of photoreceptors function in our study. Not only phototransduction is affected by hyperglycemic condition, cardiovascular supply is also diminished (Kikkawa, 2000). Sorbital and fructose are products from excessive glucose metabolism by polyol pathway. These molecules with high osmotic pressure cause damage to blood retinal barrier (Hohman et al., 1989). Furthermore, excessive amount of glucose is also metabolized through glycolysis, giving lactic acid and diacylglycerol. These glycolysis end products trigger protein kinase C pathway resulting in destruction of endothelial permeability of retinal capillaries (Jacot and Vinik, 2007).

Advanced glycation end-products are another substances present with excessive blood glucose level (Tarr et al., 2013). These substances located at retinal capillaries (Arden and Sivaprasad, 2012) are not only toxic to vessels but retinal neurons. They play a role in production of free radicals (Nishikawa et al., 2000), which is in turn causing damage to capillary permeability, vascular inflammation (Hammes et al., 1991) and leukostasis (Yamagishi, 2011). By means of retinal ischemia, growth factors such as insulin-liked growth factor, vascular endothelial growth factor are important substances stimulating new vessels formation in the retinal inner nuclear layer (Feng et al., 2007; Kern, 2007) but yet high endothelial permeability (Arden and Sivaprasad, 2012) or microaneurysm. These fragile vessels can easily lead to vessel damage, retinal hemorrhage, leukostasis and microvascular infarcts in the nerve fiber layer. In addition, massive amount of oxygen, which is essential for formation of microaneurysm particularly in the retina, causes a significant lack of oxygen supply to support normal mechanism of phototransduction (Arden and Sivaprasad, 2012). As inner retina is mainly supplied by central retinal artery (Ofri, 2008), it is severely affected in diabetic patients. Retinal vessel vasoconstriction in DM patients is caused by hypercapnia (Chung et al., 1999). Abnormal responses of the b-wave and OP, both of which are originated from the inner retina (Tzekov and Arden, 1999) are then revealed in our study.

Examination of flicker light is usually applied for a diagnosis of optic nerve damage and macular disease (Contestabile et al., 1991) in humans. Reduction of flicker responses is parallel to that of DM patients, who demonstrate low potential of retinal vessel dilatation compared to non DM patients (van Dijk et al., 2009). It is an indicative of vasoconstriction, corresponding to low amount of retinal oxygen in diabetic mice (Berkowitz et al., 2004).

Neuronal degeneration also occurs in the inner plexiform layer and nerve fiber layer (de Faria et al., 2002) of DM type 1 patients, involving microglia cells and ganglion cells (Tarr et al., 2013). Retinal hypoxia induced by hyperglycemia generates large amount of free radicals, which result in cell apoptosis. As a consequence, excessive amount of glutamate produced by apoptotic cells causes further ganglion cell death (Qian and Ripps, 2011), confirmed by Terminal dUTP Nick End Labeling (Barber et al., 2011). Abnormal ERG originated from the inner retina is even discernible (Osborne et al., 2004). In streptozotocin-induced DM rodents, large amount of tyrosin is observed near retinal blood vessels and cell bodies of ganglion cells (Qian and Ripps, 2011). Immunohistochemically, an administration of GABA receptor agonist in these DM-induced rodents had an influence on reduction of tyrosine expression, hence improving neurotransmission signals within the retina.

As located in the inner retina, amacrine cells are also severely damaged from glutamate-induced excitotoxicity (Izumi et al., 2002). It is well documented that OP is originated from amacrine cells (Wachtmeister, 1987). Similar to DM patients (Shirao and Kawasaki, 1998; Movasat et al., 2008), marked alteration of OP responses are therefore evident in our study. Due to the fact that OP response is marked reduced in DM patients, it has been used as a predictor of the onset and progression of DR (Shirao and Kawasaki, 1998; Tzekov and Arden, 1999).

Interestingly, some diabetic dogs have negative ERG waveforms, of which b/a ratio is below 1.0. This waveform characteristic is well recognized in patients with inner retinal dysfunction, especially in DM types 2 patients without retinopathy (Gualtieri et al., 2013). When b/a ratio is below 1.0 in association with appearance of negative ERG waveform, fibrous proliferation at disc or central artery occlusion is

suggested. Restricted circulation of retinal vessels may occur by central retinal occlusion or severe systemic change in the retina (Hayreh et al., 2004). Ratio of b/a is for that reason used as reliable indicator of central retinal vein occlusion (Johnson and McPhee, 1993). In dogs, negative ERG waveform is found in Briards suffered from congenital stationary night blindness (CSNB) (Narfström et al., 1989). CSNB is a genetic disorder that neuronal transmission is interrupted by lipid inclusions within the RPE (Wrigstad et al., 1992). In this study, three out of six dogs representing negative ERG waveforms had a history of diabetic ketoacidosis (DKA). Severe hyperglycemia in these dogs diagnosed with DKA leads to dehydration and hemodynamic change within blood vessels throughout the body. Retinal dysfunction is thereafter caused by lack of oxygen and nutritional supply via high viscosity of retinal blood flow.

Although fundus photography is known as a good documentation of fundus details, Early alteration of retinal vasculature in type 2 DM patients could not be detected via fundus photography (Tyrberg et al., 2011). Fluorescein angiography is much more sensitive for DR diagnosis as compared to fundus photography. It somehow fails to detect DR in type 1 DM patients (Parisi et al., 1997). Due to the reason that level of VGEF in dogs with diabetic cataract was exceedingly low to stimulate changes of retinal blood vessels (Abrams et al., 2011), either fundus photography or fluorescein angiography is still be doubtful as a diagnostic tool for DR in dogs. Especially failure of the use if fundus photography in mature diabetic cataract. According to the fact OP is considered a useful parameter to detect early DR in patients without visible retinopathy (Tzekov and Arden, 1999), significant reduction of OP responses in dogs may be used as an indicator of early retinal abnormality in DM dogs.

Conclusion

Electroretinographic responses in diabetic dogs were characterized as significant deficit in ERG amplitudes. Negative ERG waveform, together with substantial alterations of OP indicated severe retinal occlusion in ischemic retina. ERG

is a reliable indicator not only to early detect changes in the retina, but also to predict progression and severity of dogs with diabetic cataract.

Suggestions

To further investigate change in retinal blood vessels, fundus photography and fluorescein angiography would be recommended in diabetic dogs undergone cataract surgery. Because central retinal occlusion can be indicated by b/a ratio in diabetic patients, increase number of diabetic dogs may provide reliable evidence to deeply evaluate negative ERG characteristic.

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