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Date:
2013-02-01

Citation:
Kohzaki, K., Vingrys, A. J., Armitage, J. A. & Bui, B. V. (2013). Electroretinography in streptozotocin diabetic rats following acute intraocular pressure elevation. Graefe S Archive for Clinical and Experimental Ophthalmology, 251 (2), pp.529-535. https://doi.org/10.1007/s00417-012-2212-4.

Persistent Link:
https://hdl.handle.net/11343/32894

Electroretinography in streptozotocin diabetic rats following acute intraocular pressure elevation

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Notes: This study was supported by a National Health and Medical Research Council Grants 400127 (BVB) and 350224 (AJV). We the authors have full control of all primary data and they agree to allow Graefe's Archive for Clinical and Experimental Ophthalmology to review this data upon request.

Word count: total, 2662; abstract, 248; introduction, 274; methods, 1026; results, 791; discussion, 571

Number of Figures: 4

Number of Tables: 1

ABSTRACT

BACKGROUND. We consider whether pre-existing streptozotocin induced hyperglycemia in rats affects the ability of the eye to cope with a single episode of acute intraocular pressure (IOP) elevation.

METHODS. Electroretinogram **(**ERG) responses were measured (-6.08 to 1.92 log cd.s.m^{-2}) in anaesthetized (60:5 mg/kg ketamine:xylazine) dark-adapted (>12 hours) adult Sprague-Dawley rats 1 week after a single acute IOP elevation to 70 mmHg for 60 minutes. This was undertaken in rats treated 11 weeks earlier with streptozotocin (STZ, n = 12, 50 mg/kg at 6 weeks of age) or citrate buffer (n = 12). ERG responses were analyzed to derive an index of photoreceptor (a-wave), ON-bipolar (b-wave), amacrine (oscillatory potentials) and inner retinal (positive scotopic threshold response, pSTR) function.

RESULTS. One week following acute IOP elevation there was a significant reduction of the ganglion cell pSTR (-35 ± 11%, P = 0.0161) in STZ-injected animals. IN contrast the pSTR in citrate-injected animals was not significant changed (+16 ± 14%). The negative component of the STR was unaffected by this IOP elevation in either citrate or STZ-treated groups. Photoreceptoral (a-wave, citrate-control +4 ± 3%, STZ +4 ± 5%) and ON-bipolar cell (b-wave, control +4 ± 3%, STZ +4 ± 5%) mediated responses were not significantly affected by IOP elevation in either citrate- or STZ-injected rats. Finally, oscillatory potentials (citrate-control +8 ± 23%, STZ +1 ± 17%) were not reduced one week after IOP challenge.

CONCLUSIONS. The ganglion cell dominated pSTR was reduction following a single episode of IOP elevation in STZ diabetic, but not control rats. These data indicate that hyperglycemia renders the inner retina more susceptible to IOP elevation.

Keywords: electroretinogram, retina, rat, streptozotocin, hyperglycemia, diabetes, intraocular pressure

INTRODUCTION

Open-angle glaucoma is an optic neuropathy characterized primarily by a degeneration of retinal ganglion cells. Glaucoma affects over 60-million people worldwide, with some 7-million individuals blinded by this disease.[1] A major risk factor for glaucoma is elevated intraocular pressure (IOP). Although, we have current strategies to lower intraocular pressure, this treatment fails to arrest vision loss in many patients. Additionally, many glaucoma sufferers do not have high intraocular pressure. Thus other risk factors including age, family history, myopia, and diabetes are thought to modify the risk of glaucoma.[2] The relative importance of these other risk factors remains unclear. Population studies suggest that those with diabetes have a two to six fold increase in the risk of developing vision loss from glaucoma.[2, 3] However, many other trials fail to find an association between diabetes and glaucoma.[4-6]

Reduced retinal nerve fibre layer thickness has been found using ocular coherence tomography in diabetic patients.[7-10] Laboratory investigations report the presence of apoptotic ganglion cell death[10], which resembles glaucoma.[11-17] Moreover, both diabetic and glaucomatous eyes show increased levels of oxidative stress[18] and abnormalities in axonal transport. Given these similarities it is possible that diabetes modifies the risk for glaucoma.[19, 20] Few studies have directly considered whether diabetes modifies the susceptibility of the electroretinogram to acute IOP elevation. Kanamori et al.[21] found that 4 weeks after streptozotocin-injection, a common model of hyperglycemia, chronic IOP elevation led to increased apoptosis. Casson et al.[22] and Ebneter and colleague[23] report that STZ-injection protects rat eyes from IOP elevation. These above studies have not

considered the influence of STZ-induced hyperglycaemia on the response of ganglion cells a single episode of acute IOP elevation.

MATERIALS & METHODS

All experiments adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Animals

Twenty-eight Sprague-Dawley rats (6-week old, male) were used in this study. All animals were housed in an air-conditioned room (21 °C) with a 12 hours light/dark cycle (50 lux, 8 am to 8 pm). Food and water were available *ad libitum*. Animals were randomly assigned to citrate-control (c) or STZ groups (stz). Sixteen animals had hyperglycemia induced via tail vein injection of 50 mg/kg STZ (MP Biomedical, Solon, OH, USA) dissolved in trisodium citrate buffer (1 mL/kg of 0.01 M, pH 4.5; Sigma-Aldrich, Castle Hill, NSW, Aust). Twelve citrate-control animals had an equivalent volume of citrate buffer injected into the tail vein.

Blood glucose levels were measured at 1, 3, 9 and 12 weeks after treatment (Ascensia Esprit2®, Bayer HealthCare, Pymble, NSW, Aust). Animals with blood glucose levels of > 15 mmol/L were considered to be hyperglycemic. Of the 16 animals treated with STZ, 4 did not meet the above criteria and were excluded form the study.

To maintain systemic health one week after STZ treatment, diabetic animals were given 1-2 units of long-acting insulin subcutaneously every day (Protaphane®, Novo Nordisk Pharmaceuticals, Baulkham Hills, NSW, Aust). Body weight was measured weekly. At 12 weeks after citrate or STZ treatment the systemic status of animals over a 24-hour period were assessed using metabolic cages (3701M081, Techniplast Australia, Rydalmere, BC, Aust).

Acute intraocular pressure challenge

At 11 weeks after STZ or citrate treatment one randomly selected eye from each rat underwent IOP challenge for 1 hour. All rats were anesthetized with an intramuscular injection of ketamine hydrochloride (60 mg/kg; Troy Laboratories, Smithfield, NSW, Aust) and xylazine (5 mg/kg; Xylazil-100®, Troy Laboratories), which gave deep anesthesia throughout IOP elevation. Both corneas were anesthetized with 0.5% proxymetacaine hydrochloride (Alcaine®, Alcon Laboratories, Frenchs Forest, NSW, Aust) and pupils were dilated with 0.5% tropicamide (Mydriacyl®, Alcon Laboratories). The anterior chamber was cannulated with a 30-gauge needle connected by polyethelyne tubing to a pressure transducer (Transpac; Abbott Australasia, Botany, NSW, Aust) in series with a reservoir containing sterile Hanks' balanced salt solution (SAFC Biosciences, Brooklyn, VIC, Aust). IOP was monitored (Powerlab 8SP, Chart™ software; ADInstruments, Castle Hill, NSW, Aust) continuously to maintain 70 mmHg for 60 minutes. The contralateral eye in each animal was left as an untreated control (normal IOP). Blood pressure was monitored using tail-cuff sphygmomanometery (ML125R; ADInstruments) to ensure that ocular perfusion pressure was the same in both groups.[24] IOP elevated and contralateral normal IOP eyes are designated

as +IOP (+IOPc, +IOPstz) and –IOP (-IOPc, -IOPstz), respectively, with suffixes indicating whether they are citrate control or STZ-rats.

Electroretinography

Procedures for electroretinography have been described previously.[25] Briefly, light stimuli generated from white LEDs (5 Watt, Luxeon, Philips Lumileds Lighting Co. CA, USA) was delivered via a Ganzfeld sphere (Photometric Solutions International, Huntingdale, VIC, Aust). The light source was calibrated with an IL1700 photometer with a scotopic luminosity filter in place (Z-CIE, International Light Technologies Inc., Newburyport, MA, USA), to give scotopic cd.s.m^{-2}. Stimulus voltage and duration was varied (1 to 4 ms) to yield light levels ranging from -6.08 to 1.92 log cd.s.m^{-2}. Interstimulus interval was 2 seconds for the dimmest stimulus and increased progressively to 120 seconds for the bright light levels. At the dimmest light levels 20 responses were averaged, with fewer (5 - 10) averaged for moderate energies, and no averaging for bright energies (>-1 log cd.s.m^{-2}). Responses were band pass filtered 0.1 - 1000 Hz (P511, Grass Technologies, West Warwick, RI, USA) and digitized at 4 kHz (PowerLab, ADInstruments).

Electrodes were handmade from chlorided silver wire. The active electrode was placed on the center of the cornea (with lubricant eye drops Celluvisc®, Allergan, Irvine, CA, USA) and the reference was located around the equator of the same eye. The ground electrode was a stainless steel needle (Grass Technologies) inserted in the tail. Body temperature was maintained throughout the experiment at 37 ± 0.5 °C using a water heating pad.

ERG recording was performed at one week following IOP challenge, which is 12 weeks after STZ or citrate-injection. Systemic and corneal anesthesia and pupil mydriasis are as above. Responses were collected from both eyes after overnight dark-adaptation (> 12 hours).

The analysis of ERG components has been described in previous studies.[25] Briefly we analyzed the ERG for a-wave, b-wave, oscillatory potential and scotopic threshold responses (STR). The photoreceptoral response was analyzed by fitting a model (P3) based on the biochemical cascade of phototransduction,[26, 27] which gives saturated amplitude (RmP3), the sensitivity of phototransduction (S) and a delay (t_d). This model was fit to the a-wave leading edge for an ensemble of the brightest three light levels.

The P2 (ON-bipolar cell response) was analyzed after subtraction of the P3 model and band-pass filtering to remove the oscillatory potentials. The P2 amplitude as a function of light level was described using a hyperbolic function, to return the maximal amplitude (V_{max}) and the semi-saturation constant (K).

The scotopic threshold response (STR) and in particular the positive component, has been shown to reflect ganglion cell activity in rats.[28-30] The positive STR (pSTR) was evaluated by taking the amplitude at a fixed time of 120 ms after stimulus onset and by its peak implicit time. The negative STR (nSTR) was analyzed by taking the amplitude at a fixed time of 220 ms and by its trough implicit time. We also measured the oscillatory potentials of mixed and cone isolated responses (twin flash paradigm (at 1.92 log cd.s.m^{-2}, interstimulus interval 500 ms) by band-pass filtering as previous.[31, 32] OP amplitude was evaluated as the peak of the largest wavelet.

Statistical analysis

To specifically assess the IOP effect, data from IOP treated eyes was expressed relative to the contralateral control eye (% ± standard error of the mean, SEM). Normality was determined with a Kolmogorov-Smirnov test and sphericity was tested using a variance ratio. A parametric *t*-test (Prism, v5.00, GraphPad Software Inc., San Diego, CA, USA) was used to compare the IOP effect between citrate-control and STZ-treated groups. An alpha of 0.05 was applied for all statistical purposes.

RESULTS

Systemic and Metabolic changes

Table 1 shows the systemic changes (body weight, blood glucose levels and systolic blood pressure) induced by STZ-injection. At 12 weeks following treatment STZ animals had blood glucose of 25.0 ± 1.6 mmol/L as compared with citrate controls 12.8 ± 0.6 mmol/L ($P < 0.001$, Table 1). Between 3 to 12 weeks the citrate-control group showed significantly ($F_{1,1} = 18.98$, $P < 0.0001$, Table 1) greater weight gain (average increase; 119.3 ± 5.4 g) compared with the STZ group (average increase; 57.3 ± 4.0 g). STZ-treated rats also showed significantly higher food intake, water intake and waste production compared with controls ($P < 0.05$). Systolic blood pressure was not significantly different between STZ and citrate injected rats (STZ, 107.1 ± 3.5 mmHg vs citrate-controls 102.2 ± 4.6 mmHg).

Insert Table 1 and Figure 1 about here

ERG waveforms

Figure 2 shows ERG waveforms collected 1 week after IOP challenge in citrate-control (Panels A-C) and STZ (Panels D-F) rats. Panels A and C show the waveforms recorded to dim stimuli, with the STR apparent in the dimmest three intensities (-6.08 to -5.52 log cd.s.m^{-2}). Comparison of untreated to IOP-elevated eyes (Figure 2A vs 2C) shows that 12 weeks after treatment STZ animals showed relatively normal a-wave and b-waves. However, there was a reduction of the pSTR and OPs (Figure 2B vs 2D) in STZ-treated rats compared with citrate-control rats. These data are consistent with those previously reported.[25]

In STZ-rats (dim intensities in Figure 2C) the pSTR in the IOP-elevated eye was smaller than the contralateral untreated eye. In contrast IOP-elevation had little effect on the pSTR of citrated-control rats (dim intensities in Figure 2A). The nSTR showed little change in the IOP-elevated eye of itrate-control or STZ-rats.

IOP-elevation had little effect on the b-wave at moderate (-4.19 to -3.09 log cd.s.m^{-2}) to high light levels (Figure 2A and 2C) in citrate-control and STZ-rats. Figure 4B and 4D show that IOP-elevation had little effect on the OPs in citrate-control or STZ-rats.

Insert Figure 2 about here

Photoreceptor (P3) and ON-Bipolar Cell (P2) mediated responses

Figure 3A shows that IOP elevation did not significantly affect photoreceptoral P3 amplitude in citrate-control (+IOPc 555 ± 20 vs -IOPc 536 ± 20 μV) or STZ-rats (+IOPstz 560 ± 27 vs -IOPstz 554 ± 36 μV). No IOP related differences were found for phototransduction sensitivity, (+IOPc 2576 ± 128 vs -IOPc 2620 ± 139: +IOPstz 2441 ± 128 vs -IOPstz 2376 ± 109 $m^2cd^{-1}s^{-3}$) or delay (+IOPc 4.57 ± 0.07 vs –IOPc 4.54. ± 0.06 ms: +IOPstz 4.72 ± 0.06 vs –IOPstz 4.55 ± 0.07 ms).

Insert Figure 3 about here

P2 amplitude V_{max}, was not significantly affected by acute IOP-challenge in citrate-control (+IOPc; 1565 ± 49 vs -IOPc 1517 ± 54 μV) or STZ-rats (+IOPstz 1358 ± 63 vs -IOPstz 1339 ± 85 μV). Similarly, semi-saturation constant (+IOPc -2.7 ± 0.03 vs -IOPc -2.7 ± 0.04: +IOPstz -2.7 ± 0.03 vs -IOPstz -2.7 ± 0.04 log $cd.s.m^{-2}$) and slope, (+IOPc 0.8 ± 0.01 vs -IOPc; 0.8 ± 0.01; +IOPstz 0.9 ± 0.02 vs -IOPstz 0.9 ± 0.02) were not significantly affected by IOP-challenge.

Inner retinal responses

As previous,[25] the pSTR amplitude was significantly decreased in the STZ group (elevated IOP; 1.6 ± 0.6, normal IOP; 2.1 ± 0.5 μV, $P = 0.01$) compared with the citrate group (elevated IOP; 4.4 ± 0.6, normal IOP; 4.0 ± 0.4, μV).

Figure 4 shows that IOP-challenge had a significantly reduced the pSTR in STZ-rats compared with citrate-control rats (+IOPc 4.5 ± 0.6 vs –IOPc 4.0 ± 0.4: +IOPstz 1.6 ± 0.4 vs –IOPstz 2.1 ± 0.4). All other parameters were not significantly affected by IOP-elevation, including pSTR peak time (+IOPc 114 ± 2 vs -IOPc; 113 ± 1 ms: +IOPstz 113 ± 2 vs -IOPstz 108 ± 3 ms) nSTR amplitude (+IOPc -15 ± 2 vs -IOPc -16 ± 2: +IOPstz -19 ± 2 vs -IOPstz -16 ± 2 μV, $P = 0.07$) and nSTR timing (+IOPstz 224 ± 6 vs -IOPstz 228 ± 6: +IOPstz 246 ± 6 vs -IOPstz 244 ± 6 ms, $P = 0.48$).

Insert Figure 4 about here

IOP elevation did not significantly affect the amplitude of mixed (+IOPc 131 ± 20 vs -IOPc 117 ± 13: +IOPstz 94 ± 10 vs -IOPstz 92 ± 13 μV) or cone (+IOPc 22 ± 2 vs -IOPc 22 ± 2, +IOPstz 22 ± 2 vs -IOPstz 21 ± 2 μV) oscillatory potentials. Likewise, IOP elevation did not significantly affect mixed (+IOPc 36 ± 1 vs -IOPc: 35 ± 1: +IOPstz 36 ± 1 vs -IOPstz 36 ± 1 ms) or cone (+IOPc 34 ± 2 vs -IOPc; 33 ± 2: +IOPstz 36 ± 2 vs -IOPstz 35 ±2 ms) OP peak times.

DISCUSSION

Consistent with a previous study we show that 11 weeks following STZ-treatment the pSTR OPs are attenuated, whereas photoreceptoral (a-wave) or bipolar cell (b-wave) responses are not.[25] The primary finding of the current study is that IOP challenge produced a significant pSTR reduction in STZ-treated rat, but not in citrate-control animals. This effect appears to be specific to the inner retina, as ERG components arising from the outer retina were not affected

by IOP elevation. Photoreceptoral function was unaffected one week following IOP elevation. This is consistent with He et al. [33] who found complete recovery from an IOP challenge of 70 mmHg occurs within 2 hours. Like the photoreceptoral response the b-wave was not significantly affected one week following IOP elevation. It is of interest that the nSTR was not also affected more affected by IOP challenge. It is known that the nSTR in rat receives from contributions from sources other than ganglion cells.[30] Resistance to IOP elevation of other cellular contributions to the nSTR may account for the preservation of the nSTR in STZ-rats.

It is of interest that Casson et al.[22] have shown that that b-wave amplitude is protected from IOP challenge of 110 mmHg in diabetes rats 5 days and 6 weeks following STZ-induced hyperglycemia. Ebneter et al.[23] showed that chronic IOP elevation resulted in less cell loss in STZ-rats (2 to 4 weeks after STZ-injection). In contrast these studies we found that STZ-treatment lead to a small but significant increase in the susceptibility of the pSTR to IOP elevation. These different findings arisen due to differing magnitude of IOP challenge, 70 mmHg on our study compared with 110 mmHg in Casson et al.[22] It may be that the protection afforded by hyperglycemia is greater at higher levels of IOP elevation. Furthermore, we found that the increased susceptibility to IOP elevation was restricted to the pSTR, a component of the ERG that largely reflects ganglion cell integrity in rodents.[28-30] This might suggest that neuroprotection from hyperglycemia varies for different components of the ERG. Finally, hyperglycemic neuroprotection may be better over the first 6 weeks following STZ-injection,[22] and less robust at the 11 week time point used in this study. Consistent with this possibility Kanamori et al.[21] showed at 12 weeks following

STZ-injection that 8 weeks of chronic IOP elevation increased apoptosis in the retina, particularly the ganglion cell layer.

Why STZ induced hyperglycemia might increase functional susceptibility to IOP challenge is unclear. However, it is of interest that IOP challenge and diabetes produce similar changes in the eye, including abnormal glutamate recycling,[34, 35] nitric oxide regulation,[18, 36, 37] as well as biomechanical changes at the level of the optic nerve.[38] In addition, vascular endothelial cell and blood retinal barrier abnormalities in diabetic eyes [39] may reduce the capacity for diabetic eyes to autoregulation blood flow against IOP elevation. Finally, glial cell dysfunction has been demonstrated in STZ-diabetes[40] and may be a contributor to abnormal neurovascular coupling, making the vasculature less able to react to IOP elevation. The exact mechanism leading to greater ganglion cell dysfunction following IOP elevation in STZ-treated rats needs to be further investigated.

Conclusions

One week following a single episode of moderate IOP challenge inner retinal function as measured using the ERG was reduced in STZ-treated rat eyes. This reduction was not noted in the citrate-control cohort. This outcome suggests that inner retinal function in diabetic eyes is more susceptible to IOP elevation.

Acknowledgements

Supported by a National Health and Medical Research Council Grant 400127 (BVB).

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FIGURE CAPTIONS

FIGURE 1. Metabolic indices for citrate and STZ-treated rats 11 weeks after STZ treatment. Values represent mean ± SEM (citrate unfilled; n = 4, STZ filled; n = 6). * $P < 0.05$, ** $P < 0.01$ compared with controls.

FIGURE 2. The effect of IOP elevation (70 mmHg for 60 minutes) on the full-field ERG waveforms at 11 weeks after STZ treatment. (A and B) Citrate-control group waveforms from the uncannulated normal IOP (thin traces) and the elevated IOP eye (bold traces). (C and D) STZ group waveforms from the normal IOP (thin traces) and the elevated IOP eye (bold traces). A and C show raw ERG waveforms (-5.68 to 1.92 log cd.s.m^{-2}). The dimmest flashes show the scotopic threshold responses. B and D show isolated oscillatory potentials isolated following filtering for the brighter stimuli.

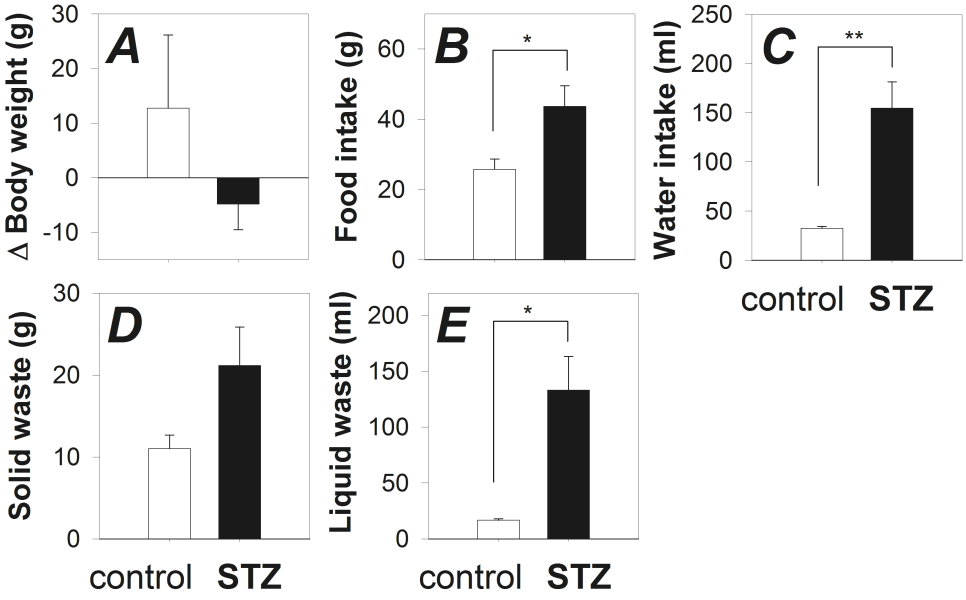
FIGURE 3. The effect of IOP elevation on the photoreceptor P3 and ON-bipolar cell P2 parameters at 11 weeks after citrate (unfilled) or STZ-treatment (filled). (A) Saturated photoreceptor amplitude (Rm_{P3}), sensitivity (S), and delay (td) in the both groups. (B) Maximal amplitude of the P2 (V_{max}), semi-saturation constant (K), and the slope (n) in both groups. Data are normalized to the uncannulated normal IOP eye (IOP eye / normal eye, %) and shown as mean ± SEM in citrate (filled, n = 12) and STZ rats (filled, n = 12). Dashed lines indicate no difference (100%). Statistical significance was evaluated by an unpaired t-test.

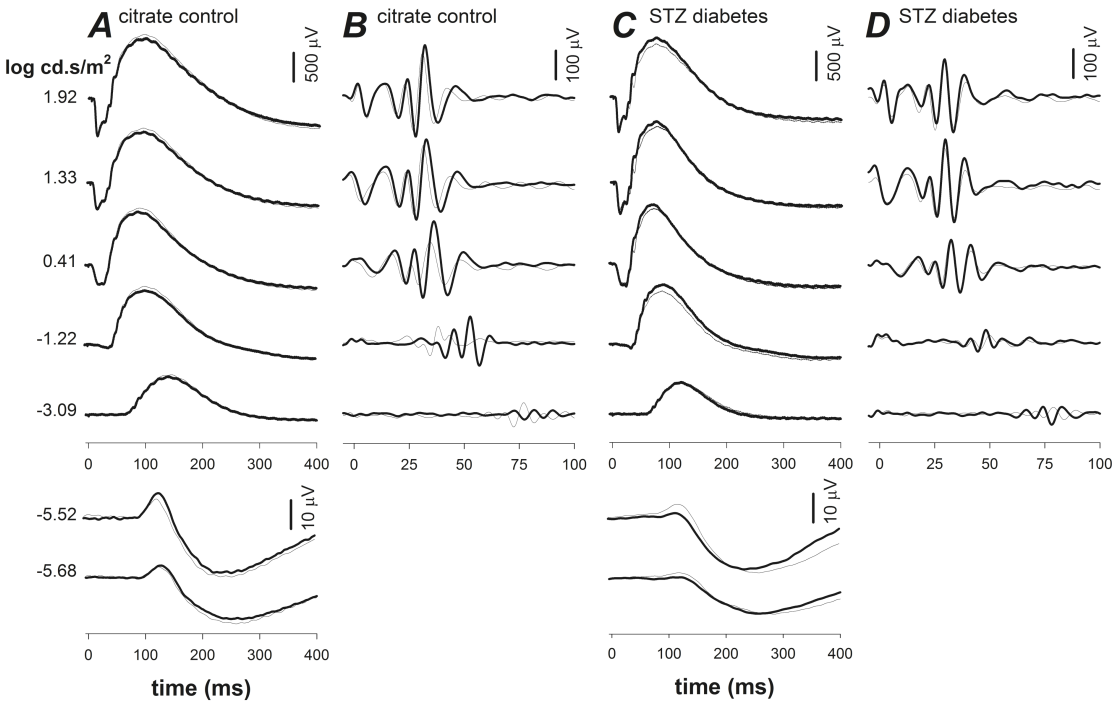
FIGURE 4. The effect of IOP elevation (70 mmHg for 60 minutes) on inner retinal function following STZ treatment at 11 weeks. Panel A presents the inner retinal amplitude such as pSTR, nSTR, mixed oscillatory potentials, and cone oscillatory potentials. Panel B also shows the peak times of each response. Data are normalized to the uncannulated normal IOP eye (IOP eye /normal eye, %) and shown as mean ± SEM for controls (unfilled, n = 12) and STZ rats (filled, n = 12). Dashed lines indicate no difference (100%). Statistical significance was evaluated by an unpaired t-test. * $P < 0.05$ compared with controls.

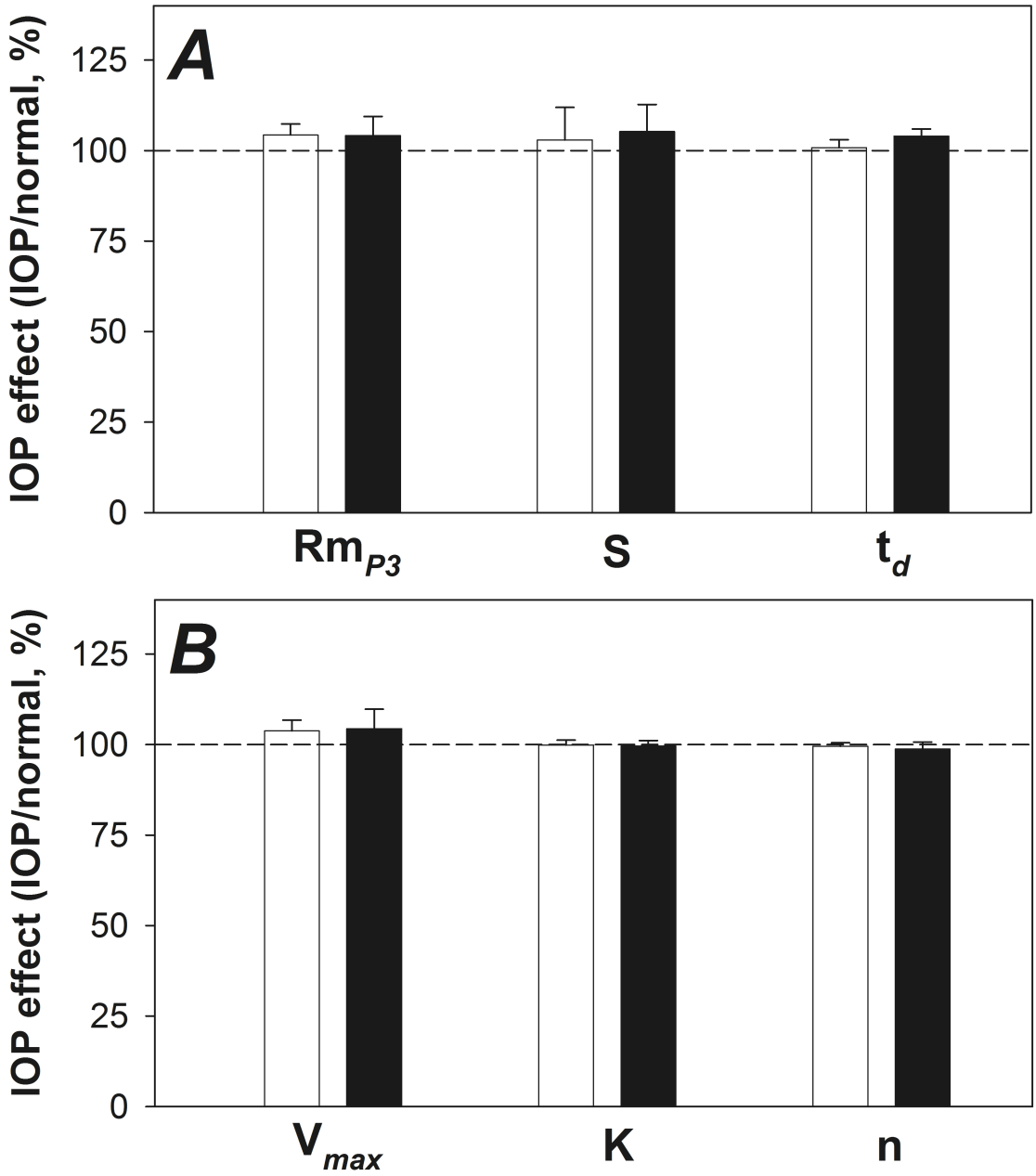
TABLE 1. Relationship with the systemic changes between controls versus STZ induced rats.

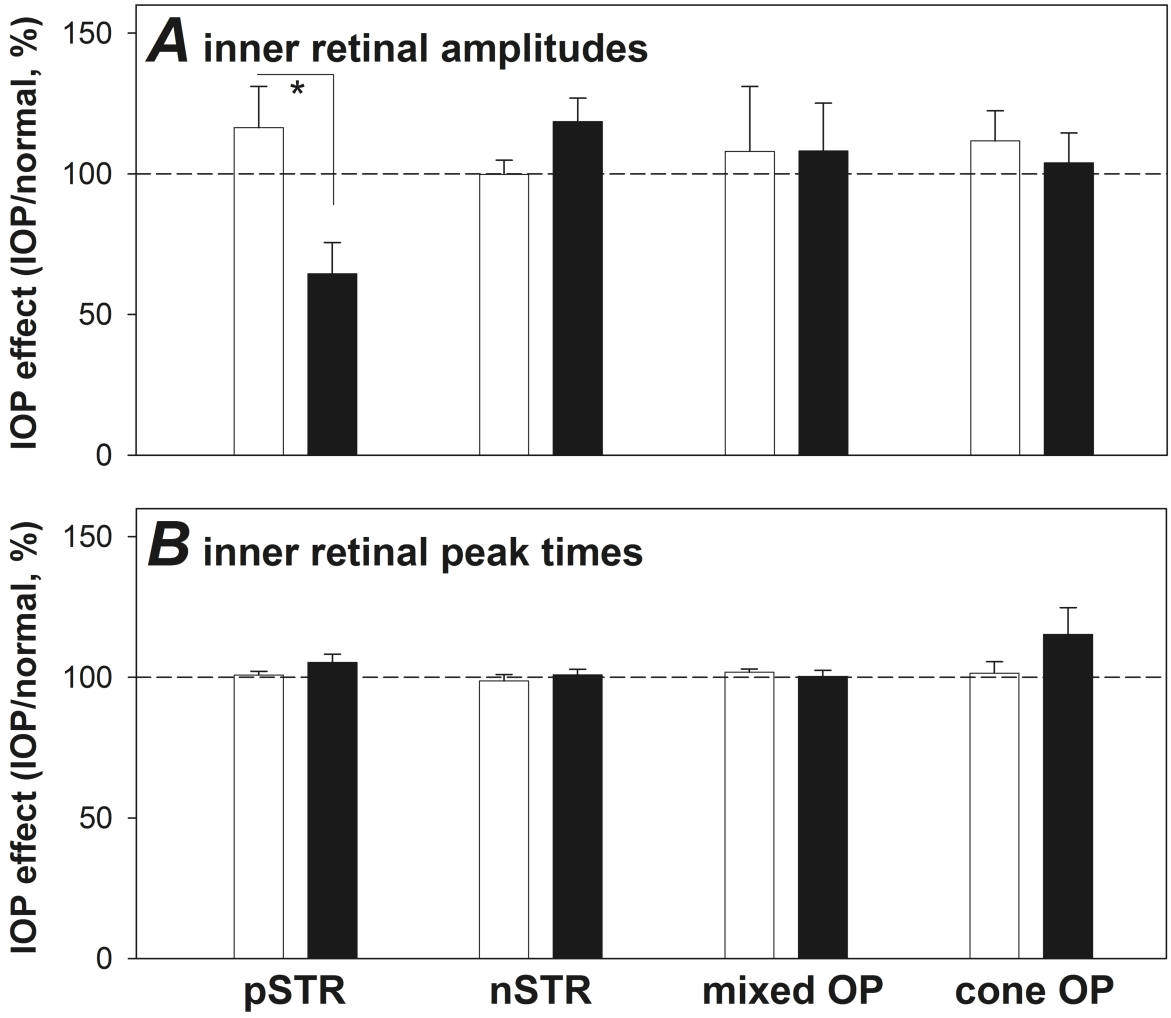
Animal	**control**	**STZ**	***P* value**
Numbers	12	12	
Weeks	12	12	
Systemic changes			
Body Weight Change (g)	+119.3 ± 5.4	+57.3 ± 4.0	< 0.001
Average Blood Glucose (mmol/L)	12.8 ± 0.6	25.0 ± 1.6	< 0.001
Average Systolic Blood Pressure (mmHg)	102.2 ± 4.6	107.1 ± 3.5	0.4051

Values present as mean ± SEM. Statistical significance was evaluated by ANOVA and unpaired *t*-test. Systemic changes; control versus STZ (n = 12, respectively). Weeks; time after STZ treatment. *P* value compared with controls. *4 were excluded as blood glucose was < 15 mmol/L.


A
Δ Body weight (g)
30
20
10
0
-10
B
Food intake (g)
60
40
20
0
*
C
Water intake (ml)
250
200
150
100
50
0
**
control
STZ
D
Solid waste (g)
30
20
10
0
control
STZ
E
Liquid waste (ml)
200
150
100
50
0
*
control
STZ


A
citrate control
B
citrate control
C
STZ diabetes
D
STZ diabetes
log cd.s/m²
1.92
1.33
0.41
-1.22
-3.09
-5.52
-5.68
500 µV
100 µV
10 µV
0
100
200
300
400
25
50
75
time (ms)


A
IOP effect (IOP/normal, %)
125
100
75
50
25
0
Rm$_{P3}$
S
t$_d$
B
IOP effect (IOP/normal, %)
125
100
75
50
25
0
V$_{max}$
K
n


A inner retinal amplitudes
IOP effect (IOP/normal, %)
150
100
50
0
*
B inner retinal peak times
IOP effect (IOP/normal, %)
150
100
50
0
pSTR
nSTR
mixed OP
cone OP

Author/s:
Kohzaki, Kenichi; Vingrys, Algis J.; Armitage, James A.; Bui, Bang V.

Title:
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Date:
2013

Citation:
Kohzaki, K., Vingrys, A. J., Armitage, J. A. & Bui, B. V. (2013). Electroretinography in streptozotocin diabetic rats following acute intraocular pressure elevation. Graefe's Archive for Clinical and Experimental Ophthalmology, 251(2), 529-535.

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