Differences in the Effects of Pentobarbital Anesthetic and Combination of Medetomidine Hydrochloride, Midazolam, and Butorphanol Tartrate Anesthetic on Electroretinogram in Spontaneously Diabetic Torii Fatty Rats

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Abstract
Purpose: The aim of this study was to investigate the effects of different anesthetic agents on electroretinograms (ERGs) in Spontaneously Diabetic Torii fatty rats (SDT fatty rats). ***Methods:*** The ERG recordings were measured under general anesthesia using pentobarbital or a combination of medetomidine hydrochloride, midazolam, and butorphanol (MMB) tartrate anesthesia in 12 9-week-old normal Sprague-Dawley rats (Jcl:SD rats) and 16 SDT fatty rats. Each animal model was divided into 2 groups, the pentobarbital group and MMB group. The amplitudes and peak times of the a- and b-waves and oscillatory potentials (OPs) were measured from 0.0001 candela per square meter (cd.s/m^2) to 10.0 cd.s/m^2. ***Results:*** The amplitude of the a-wave was significantly higher in the MMB group of Jcl:SD rats, but there was no significant difference in amplitude between the two groups of SDT

fatty rats. There was no significant difference in the OP1 amplitude between both groups of Jcl:SD rats, but the OP1 amplitude was significantly higher in the MMB group of SDT fatty rats. The OP2 amplitude was significantly higher in the pentobarbital group in both the Jcl:SD rats and SDT fatty rats. There was no significant difference in the OP3 amplitude between the Jcl:SD and SDT fatty rat groups. The amplitude of the OP4 waves was significantly higher in the MMB group for both Jcl:SD and SDT fatty rats. There was no significant difference in the sums of the OP1 to OP4 (ΣOPs) amplitudes between the Jcl:SD and SDT fatty rat groups. There was no significant difference in the b-wave amplitude between the Jcl:SD rat groups, but the b-wave amplitude was significantly higher in the SDT fatty rats that received pentobarbital. The peak times for a-wave, OP1, OP2, OP3, OP4, and ΣOPs were significantly longer in the pentobarbital group of SD rats. The peak time of the b-wave was significantly longer in the MMB group of Jcl:SD rats, but the same result was obtained in the SDT fatty rats except that there was no significant difference in the a-wave. ***Conclusion:*** The overall ERG results vary depending on the anesthetic agent used. The

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OPs can be observed in detail when using MMB. Since the SDT fatty rat is a diabetic model animal, we recommend MMB as the anesthesia of choice when studying the OP waves in detail.

Introduction

Diabetic retinopathy (DR) is a major cause of visual impairment and blindness in developed countries [1]. The pathogenesis, preventive methods, and treatments should be studied in animal models; however, an animal model of diabetes with ocular complications mimicking human diabetics should be developed.

Goto-Kakizaki rats, nonobese animals with mild type 2 diabetes [2, 3], develop retinal angiogenesis at 6 and 7 months of age [4], and electroretinograms (ERGs) show photoreceptor dysfunction in these rats [5]. Long-Evans Tokushima Lean rats have been used as a model of type 1 diabetes. Yang et al. [6] reported that the retinas of Otsuka Long-Evans Tokushima Fatty rats, a well-known model of type 2 diabetes, were significantly thinner than in the normal Long-Evans Tokushima Otsuka rats, a tendency that was apparent in the retinal nerve fiber layer using spectral-domain optical coherence tomography. Neither rat exhibited the same changes as humans in the fundus tissue. The findings especially differed from diabetic macular edema, and it was impossible to confirm the retinal thickening in most of the animal models.

A spontaneously type 2 diabetic strain of the SD rat, the Spontaneously Diabetic Torii (SDT) rat, was established in 1997 [7] and exhibited hyperglycemia, nephropathy, and peripheral neuropathy [8]. We reported severe diabetic ocular complications in this model [9–12], i.e., mature diabetic cataract and severe DR. The development of severe retinopathy with retinal edema is a major difference from the retinopathy that occurs in other diabetic animal models.

A disadvantage of the SDT rat model is that DR develops over an extended period, i.e., 80% of 51- to 60-week-old SDT rats have severe DR [10]. In addition, a number of animals die spontaneously before severe DR develops, which underscored the need for a model that developed early onset DR.

The SDT fatty rat, established in 2004 with the introduction of the fa allele (obesity gene) of the Zucker fatty rat into the SDT rat genome, is a relatively new model of obese type 2 diabetes. The prominent findings of hyperglycemia, overt obesity, hyperlipidemia, and diabetes-related complications develop at a younger age in the SDT fatty rats compared to SDT rats [13, 14], making the SDT fatty rats the best animal model of DR.

The ERG is an effective means to evaluate retinopathy, and it is an objective retinal function test that can be performed even in cataractous eyes. ERGs require general anesthesia in animals, and several anesthetic agents are available; diethyl ether inhalation anesthesia, pentobarbital sodium, and the combination of medetomidine hydrochloride, midazolam, and butorphanol tartrate (MMB) have been used widely. However, since diethyl ether inhalation anesthesia may affect the examiner via inhalation, its use is limited. Pentobarbital sodium has a hypnotic effect and is effective for measuring ERGs, but it is associated with the risk of sudden death during anesthesia. Some studies have reported it affects the oscillatory potential (OP) waves in murine [15] and canine [16] ERGs.

In the case of diabetes, the first change in the ERG occurs in the OP wave [17], which is thought to originate from the cells in the inner nuclear layer of the retina, especially the amacrine cells. The OP has been reported to be sensitive to circulatory disorders [17]. In addition to diabetes, it has been reported that OP waves are attenuated in Takayasu disease and Behcet's disease [18]. The purpose of this study was to investigate how different anesthetic agents affect ERGs in SDT fatty rats.

Materials and Methods

The care and handling of animals were in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research and the Guidelines for Animal Experimentation of the Animal Care and Committee of Jichi Medical University, the latter of which approved all experiments (17105-02). All breeding and experiments were conducted at the Saitama Medical Center Jichi Medical University and Animal Laboratory according to the In Vivo Experiments guidelines for the use of animals in research. We used 16 male SDT fatty rats (disease group) and 12 male Sprague-Dawley (Jcl:SD) rats (control group) (CLEA Japan Inc., Tokyo, Japan). Each rat was shipped to our facility in February 2019, 6 weeks after birth. The SDT fatty rats were diagnosed with diabetes by 8 weeks after birth because all rats were confirmed to be diabetic based on a nonfasting blood glucose concentration exceeding 350 mg/dL. All rats were fed standard rat chow (CRF-1, Oriental Yeast Inc., Tokyo, Japan). The breeding environment was controlled at 22° C, and 2 animals were bred in one cage. In addition, the room was brightly illuminated from 6:00 a.m. to 5:59 p.m. and dark from 6:00 p.m. to 5:59 a.m. SDT fatty rats ($n = 16$) and Jcl:SD rats ($n = 12$) were randomly divided into the pentobarbital group (SDT fatty rats: $n = 8$, Jcl:SD rats: $n = 6$) and the MMB group (SDT fatty rats: $n = 8$, Jcl:SD rats: $n = 6$). In our study, deaths during anesthesia were excluded from the analysis, but none occurred.
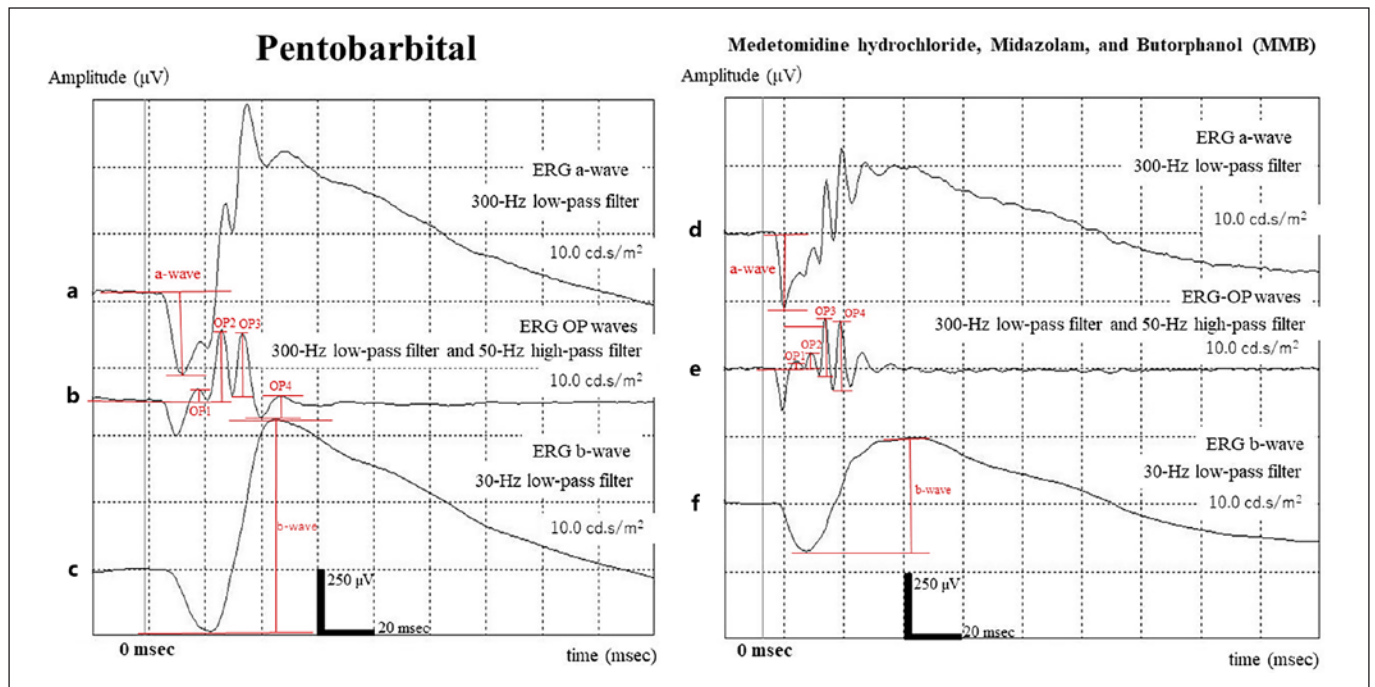
Fig. 1. ERGs analysis and measurement method. **a** Representative ERG recordings performed at 10.0 cd.s/m² using MMB. The waveforms were processed with a 300-Hz low-pass filter, mainly used for a-wave analysis. **b** The waveforms were processed with a 300-Hz low-pass filter and 50-Hz high-pass filter, mainly used for OP wave analysis. **c** The waveforms were processed with a 30-Hz low-pass filter, mainly used for b-wave analysis. **d** Representative ERG recordings performed at 10.0 cd.s/m² using pentobarbital. The waveforms were processed with a 300-Hz low-pass filter, mainly used for a-wave analysis. **e** The waveforms were processed with a 300-Hz low-pass filter and 50-Hz high-pass filter mainly used for OP wave analysis. **f** The waveforms were processed with a 30-Hz low-pass filter, mainly used for b-wave analysis.

The MMB combination anesthetic agent contained midazolam (2.0 mL/kg, Sandoz, Yamagata, Japan), medetomidine (0.375 mL/kg, Nippon Zenyaku Kogyo Co., Fukushima, Japan), and butorphanol tartrate (2.5 mL/kg, Meiji Seika Pharma, Tokyo, Japan). MMB is prepared by mixing these drugs with physiologic saline. We administered 0.5 mL of purified MMB per 100 g of the rat body weight by intraperitoneal administration [19]. If a significant anesthetic effect was not obtained with the initial dose of 0.5 mL/100 g, we increased the dose by 20% (e.g., body weight 600 g = initial amount of anesthetic, 3.0 mL, followed by additional amount of anesthesia 0.6 mL). After completion of the ERG recording, 0.5 mL of medetomidine antagonist per 100 g of rat body weight was administered to awaken the animals. Pentobarbital sodium was applied at the lowest effective dose (1 mL/kg, Kyouritu Co., Ltd., Tokyo, Japan). Pentobarbital is often administered intraperitoneally at 30 mg/kg for surgery such as pituitary gland removal and ovariectomy and 40 mg/kg for central depression [20]. In this experiment, a 30- to 40-mg/kg dose of pentobarbital had no effect. The lethal dose is generally said to be 75 mg/kg. Care should be taken when giving additional doses. When the first pentobarbital dose of 1 mL/kg was not effective, we increased the dose by 20% (e.g., body weight 359 g = initial amount of anesthetic 3.0 mL, followed by an additional 0.07 mL). When the effect of anesthesia was sustained from the ERG test, the body temperature was controlled using a warming device. A 60–90 min sleep effect is achieved by using these two methods.

Full-field ERG responses were recorded using a Ganzfeld dome, an acquisition system, and light-emitting diode stimulators (PuREC, MAYO Corporation, Inazawa, Japan). The ERG was recorded at 9 weeks of age after overnight dark adaptation. In addition, the animals fasted before general anesthesia. General anesthesia was performed under dim red light. To achieve mydriasis, a mixture of 0.5% phenylephrine hydrochloride and 0.5% tropicamide (Santen Pharmaceutical Co., Osaka, Japan) was used. The active electrodes were applied to the eyes using contact lenses, and the reference electrodes were placed in the mouth of the animals. An electrode clipped to the tail served as the ground. The ERG response, measured from 0.0001 candela per square meter (cd.s/m²) to 10.0 cd.s/m², was obtained from the left eye of each rat.

The ERG a-waves were analyzed after processing with a 300-Hz low-pass filter (Fig. 1a, d). The OP waves were analyzed after processing with a 300-Hz low-pass filter and 50-Hz high-pass filter (Fig. 1b, e). ERG b-waves were analyzed after processing with a 30-Hz low-pass filter (Fig. 1c, f). Four OP waves were confirmed; the amplitude of OP1 was measured from the baseline and those of OP2, OP3, and OP4 were measured from the lowest point of the last negative wave to the peak of each waveform. The peak times of the OPs were measured from the tops of the OPs. The total OPs were expressed as ΣOPs, which is the sum of OP1 to OP4. The amplitude and peak time of each ERG waveform, rat body weight, and anesthesia dose were measured and analyzed.

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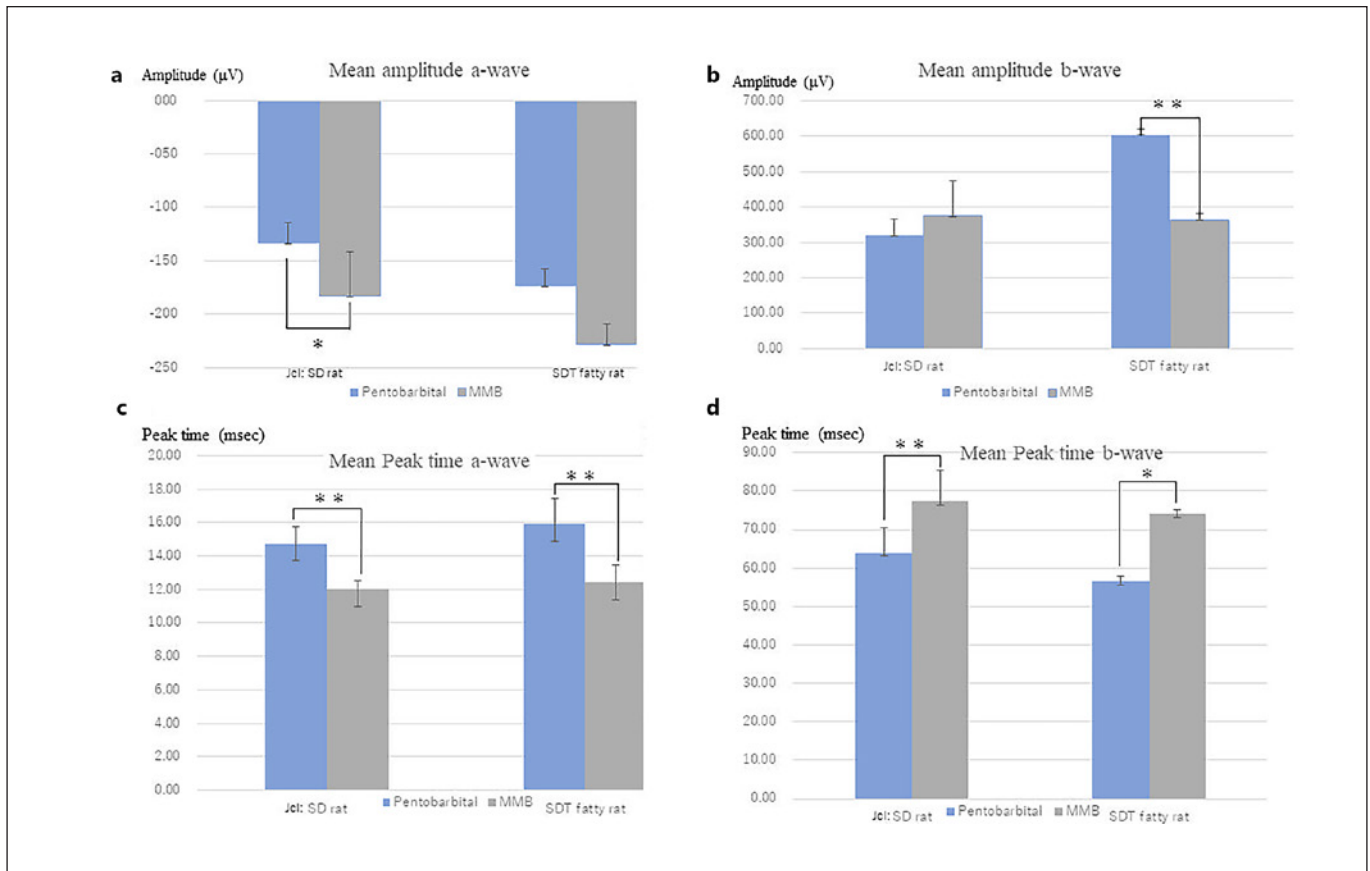
Fig. 2. Comparison of ERG a-waves and b-waves in the Jcl:SD rats and SDT fatty rats treated with pentobarbital and MMB groups. The ERGs were measured in all rats at 9 weeks. **a** The a-wave was significant in the Jcl:SD rats (Mann-Whitney *U*-test **p* = 0.0104) but not in the SDT fatty rats (Mann-Whitney *U*-test *p* = 0.5286). **b** The b-wave amplitude was not significant in the Jcl:SD rats (Mann-Whitney *U*-test *p* = 0.3367) but was significantly higher in the MMB group in the SDT fatty rats (Mann-Whitney *******p* = 0.0063). **c** A comparison of the a-wave peak times in the MMB and pentobarbital groups shows that the a-wave peak times are significantly longer in the pentobarbital group of Jcl:SD rats (Mann-Whitney *U*-test **p = 0.0039) and SDT fatty rats (Mann-Whitney *U*-test **p = 0.0063). **d** A comparison of the b-wave peak times in the MMB and pentobarbital groups shows that the b-wave peak times are significantly longer in the MMB group of Jcl:SD rats (Mann-Whitney *U*-test **p = 0.0025) and SDT fatty rats (Mann-Whitney *U*-test **p* = 0.0209).

All statistical analyses were performed using Excel Tokei 2006 software (Social Survey Research Information Co. Ltd., Tokyo, Japan). All data are expressed as the mean ± standard deviation. The data from each ERG waves amplitude and peak time were compared using the Mann-Whitney *U*-test. *p* < 0.05 was considered statistically significant.

Results

The ERGs were measured in 8 animals each in the pentobarbital and MMB groups at 9 weeks of age. The average body weight of the pentobarbital group of Jcl:SD rats was 363.015 ± 9.44 g and that of the MMB group of Jcl:SD rats was 402.4625 ± 15.9476 g. The average body weight of the pento-

barbital group of SDT fatty rats was 363.01 ± 9.45 g and that of the MMB group of SDT fatty rats was 402.4625 ± 15.94 g.

The average anesthesia dose in the pentobarbital group of Jcl:SD rats was 0.365 ± 0.003 mL and 2.1 ± 0.122 mL in the MMB group of Jcl:SD rats. No Jcl:SD rats were given additional doses without anesthesia.

The average anesthesia dose in the pentobarbital group of SDT fatty rats was 0.365 ± 0.009 mL and 2.0375 ± 0.07 mL in the MMB group of SDT fatty rats. When 1 animal in the pentobarbital group did not respond to the anesthesia, the dose was increased by 0.07 mL. When 3 animals in the MMB group did not respond to the anesthesia, the dose was increased by 0.4–2.0 mL [20]. No rats died due to the increased doses in either group.
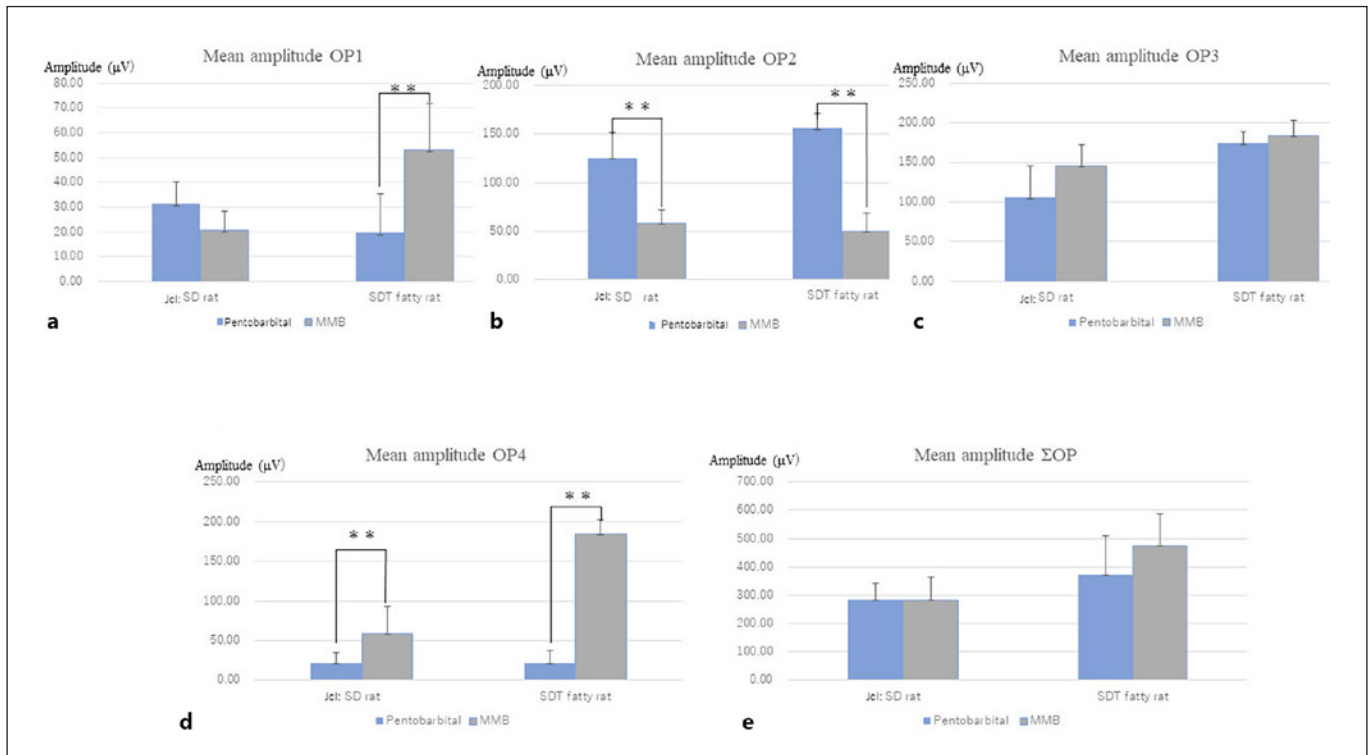
Fig. 3. Comparison of ERG OP amplitudes of the Jcl:SD rats and SDT fatty rats treated with pentobarbital and MMB. All rats measured ERG at 9 weeks. **a** The OP1 amplitude was not significant in the Jcl:SD rat (Mann-Whitney U-test $p = 0.0547$) but was significantly higher in the MMB group in SDT fatty rats (Mann-Whitney U-test **$p = 0.0046$). **b** A comparison of the OP2 amplitudes in the MMB and pentobarbital groups shows that they are significantly higher in the pentobarbital group of Jcl:SD rats (Mann-Whitney U-test **$p = 0.0065$) and SDT fatty rat (Mann-Whitney U-test **$p = 0.0033$). **c** There was no significant difference in OP3 amplitude between the Jcl:SD rats (Mann-Whitney U-test $p = 0.1093$) and SDT fatty rats (Mann-Whitney U-test $p = 0.9164$). **d** A comparison of the OP4 amplitudes in the MMB and pentobarbital groups shows that the amplitudes are significantly higher in the MMB group of Jcl:SD rats (Mann-Whitney U-test **$p = 0.0025$) and SDT fatty rats (Mann-Whitney U-test **$p = 0.0008$). **e** There was no significant difference in the ΣOP amplitudes between the Jcl:SD rats (Mann-Whitney U-test $p = 0.8728$) and SDT fatty rats (Mann-Whitney U-test $p = 0.1152$).

The a-wave amplitude was significantly higher in the MMB group of Jcl:SD rats (pentobarbital group, -133.7 ± 19.23 μV vs. MMB group, -182.5 ± 41.09 μV, $p = 0.00104$) (Fig. 2a). No significant differences in the OP1 amplitudes were seen between both groups of Jcl:SD rats (pentobarbital group, 31.5 ± 8.5 μV vs. MMB group, 20.7 ± 7.71 μV, $p = 0.0547$) (Fig. 3a). The OP2 amplitude was significantly higher in the pentobarbital group of Jcl:SD rats (pentobarbital group, 125.3 ± 26.3 μV vs. MMB group, 58.5 ± 12.8 μV, $p = 0.0065$) (Fig. 3b). No significant differences in the OP3 amplitudes were seen between both groups of Jcl:SD rats (pentobarbital group, 105.1 ± 40.37 μV vs. MMB group, 145.2 ± 26.73 μV, $p = 0.1093$) (Fig. 3c). The OP4 wave amplitude was significantly higher in the MMB group of Jcl:SD rats (pentobarbital group, 22.1 ± 12.16 μV vs. MMB group, 58.7 ± 34.86 μV, $p = 0.025$) (Fig. 3d). No

significant differences in the ΣOP amplitudes were seen between both groups of Jcl:SD rats (pentobarbital group, 283.9 ± 60.17 μV vs. MMB group, 283.1 ± 78.96 μV, $p = 0.8728$) (Fig. 3e). No significant differences in the b-wave amplitudes were seen between both groups of Jcl:SD rats (pentobarbital group, 320.4 ± 45.72 μV vs. MMB group, 374.1 ± 99.13 μV, $p = 0.3367$) (Fig. 2b).

The peak time of the a-wave was significantly longer in the pentobarbital group of Jcl:SD rats (pentobarbital group, 14.7 ± 1.08 ms vs. MMB group, 12.0 ± 0.51 ms, $p = 0.0039$) (Fig. 2c). The peak time of the OP1 was significantly longer in the pentobarbital group of Jcl:SD rats (pentobarbital group, 20.5 ± 0.73 ms vs. MMB group, 18.2 ± 0.67 ms, $p = 0.0039$) (Fig. 4a). The peak time of the OP2 was significantly longer in the pentobarbital group of Jcl:SD rats (pentobarbital group, 29.0 ± 1.49 ms vs. MMB
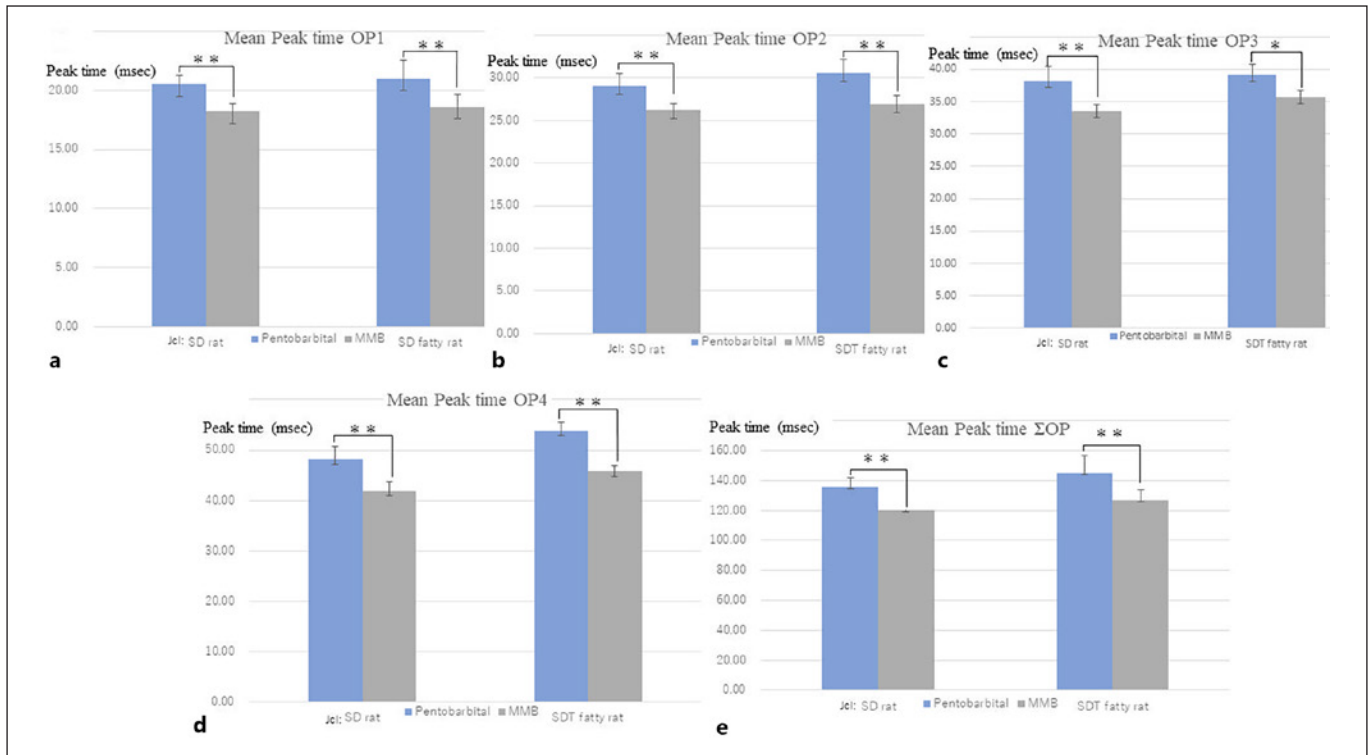
Fig. 4. Comparison of ERG OP peak time of pentobarbital and MMB in Jcl:SD rats and SDT fatty rats. The ERGs were recorded in all rats at 9 weeks of age. **a** A comparison of the OP1 peak times in the MMB and pentobarbital groups shows that the OP1 peak times are significantly longer in the pentobarbital group of Jcl:SD rats (Mann-Whitney *U*-test **$p = 0.0039$) and SDT fatty rats (Mann-Whitney *U*-test **$p = 0.0023$). **b** A comparison of the OP2 peak times in the MMB and pentobarbital groups shows that the OP peak times are significantly longer in the pentobarbital group of Jcl:SD rats (Mann-Whitney *U*-test **$p = 0.0082$) and SDT fatty rats (Mann-Whitney *U*-test **$p = 0.0016$). **c** A comparison of the OP3 peak times in the MMB and pentobarbital groups shows that the OP3 peak times are significantly longer in the pentobarbital group of Jcl:SD rats (Mann-Whitney *U*-test **$p = 0.0039$) and SDT fatty rats (Mann-Whitney *U*-test *$p = 0.0274$). **d** A comparison of the OP4 peak times in the MMB and pentobarbital groups shows that the OP4 peak times are significantly longer in the pentobarbital group of Jcl:SD rats (Mann-Whitney *U*-test **$p = 0.0051$) and SDT fatty rats (Mann-Whitney *U*-test **$p = 0.0046$). **e** A comparison of the OP4 peak times in the MMB and pentobarbital groups shows that the ΣOP peak times are significantly longer in the Jcl:SD rats (Mann-Whitney *U*-test **$p = 0.0039$) and the SDT fatty rats (Mann-Whitney *U*-test **$p = 0.0039$) treated with pentobarbital.

group, 26.2 ± 0.7 ms, $p = 0.0082$) (Fig. 4b). The peak time of the OP3 was significantly longer in the pentobarbital group of Jcl:SD rats (pentobarbital group, 38.2 ± 2.23 ms vs. MMB group, 33.5 ± 0.99 ms, $p = 0.0039$) (Fig. 4c). The peak time of the OP4 was significantly longer in the pentobarbital group of Jcl:SD rats (pentobarbital group, 48.2 ± 2.46 ms vs. MMB group, 41.9 ± 1.73 ms, $p = 0.0051$) (Fig. 4d). The peak time of the ΣOPs was significantly longer in the pentobarbital group of Jcl:SD rats (pentobarbital group, 135.8 ± 5.76 ms vs. MMB group, 120.1 ± 3.81 ms, $p = 0.0039$) (Fig. 4e). The peak time of the b-wave was significantly longer in the MMB group of Jcl:SD rats (pentobarbital group, 64.0 ± 6.3 ms vs. MMB group, 77.2 ± 8.26 ms, $p = 0.025$) (Fig. 2d).

No significant differences in the a-wave amplitudes were seen between both groups of SDT fatty rats (pentobarbital group, -173.3 ± 125.25 μV vs. MMB group, -227.9 ± 49.46 μV, $p = 0.5286$) (Fig. 2a). The OP1 amplitude was significantly higher in the MMB group of SDT fatty rats (pentobarbital group, 15.9 ± 19.73 μV vs. MMB group, 53.2 ± 18.74 μV, $p = 0.0046$) (Fig. 3a). The OP2 amplitude was significantly higher in the pentobarbital group of SDT fatty rats (pentobarbital group, 156.0 ± 61.26 μV vs. MMB group, 49.9 ± 13.64 μV, $p = 0.0033$) (Fig. 3b). There was no difference in the OP3 amplitudes between both groups of SDT fatty rats (pentobarbital group, 173.7 ± 69.47 μV vs. MMB group, 183.9 ± 50.73 μV, $p = 0.9164$) (Fig. 3c). The OP4 amplitude was significantly higher in the MMB
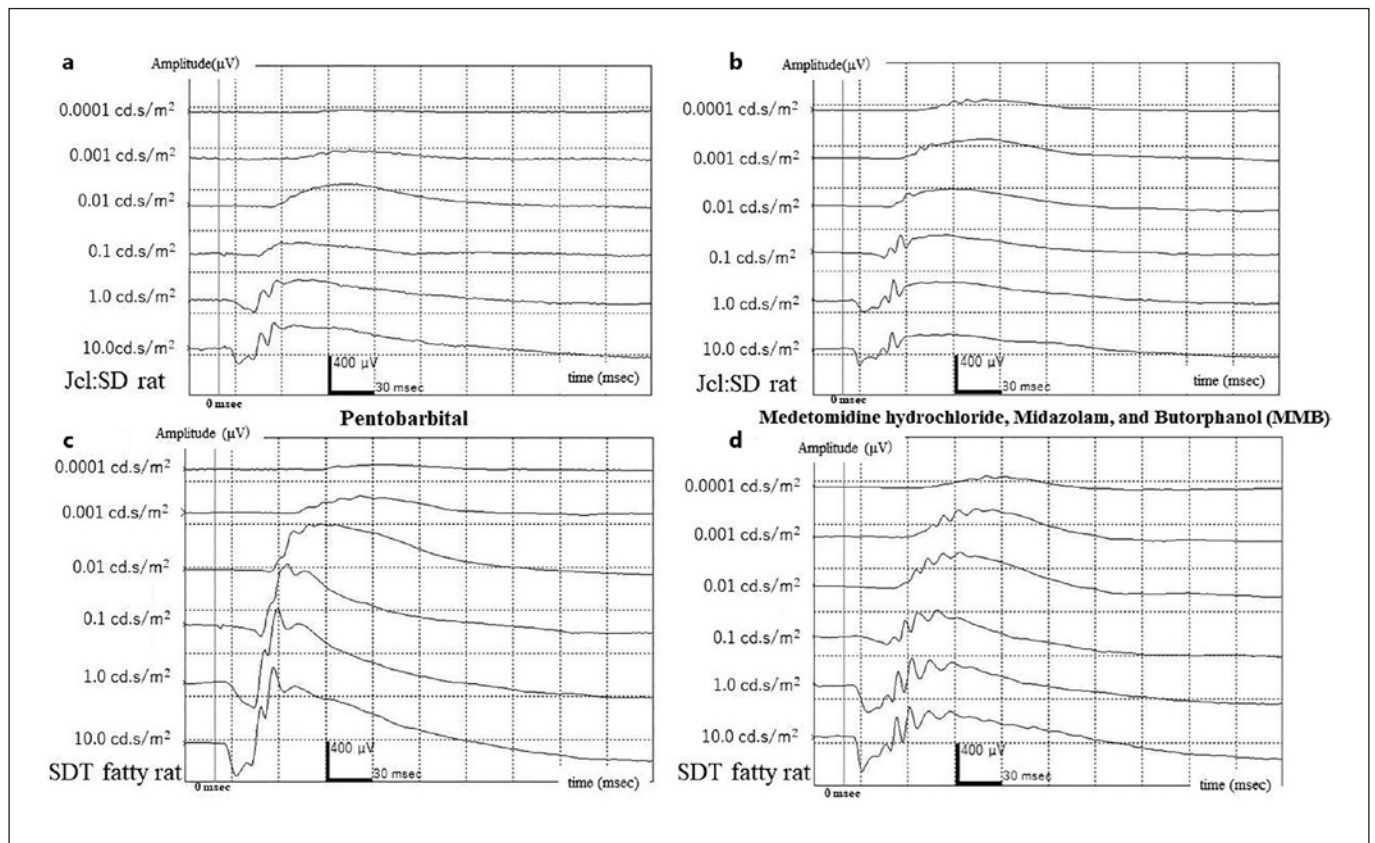
Fig. 5. ERG waveforms associated with changes in light intensity. **a** The changes in the ERG waveform when pentobarbital is used in Jcl:SD rats. **b** The changes in the ERG waveform when MMB is used in Jcl:SD rats. The b- and OP waves are observed at 0.0001 cd.s/m2 in the MMB group. **c** The changes in the ERG waveform when pentobarbital is used in SDT fatty rats. **d** The changes in the ERG waveform when MMB is used in SDT fatty rats. The b- and OP waves are observed at 0.0001 cd.s/m^2 in the MMB group.

group of SDT fatty rats (pentobarbital group, 22.0 ± 15.54 µV vs. MMB group, 188.3 ± 52.09 µV, $p = 0.0008$) (Fig. 3d). There was no difference in the ΣOPs amplitudes between both groups of SDT fatty rats (pentobarbital group, 371.2 ± 136.69 µV vs. MMB group, 475.4.9 ± 112.14 µV, $p = 0.1152$) (Fig. 3e). The b-wave amplitude was significantly higher in the pentobarbital group of SDT fatty rats (pentobarbital group, 604.3 ± 181.03 µV vs. MMB group, 362.9 ± 82.66 µV, $p = 0.0063$) (Fig. 2b).

The peak time of the a-wave was significantly longer in the pentobarbital group of SDT fatty rats (pentobarbital group, 15.9 ± 2.28 ms vs. MMB group, 12.4 ± 0.43 ms, $p = 0.0063$) (Fig. 3c). The peak time of OP1 was significantly longer in the pentobarbital group of SDT fatty rats (pentobarbital group, 21.0 ± 1.54 ms vs. MMB group, 18.6 ± 1.03 ms, $p = 0.0023$) (Fig. 4a). The peak time of OP2 was significantly longer in the pentobarbital group of SDT fatty rats (pentobarbital group, 30.6 ± 2.49 ms vs. MMB

group, 26.9 ± 1.4 ms, $p = 0.0016$) (Fig. 4b). The peak time of OP3 was significantly longer in the pentobarbital group of SDT fatty rats (pentobarbital group, 39.2 ± 3.24 ms vs. MMB group, 35.7 ± 2.45 ms, $p = 0.0274$) (Fig. 4c). The peak time of OP4 was significantly longer in the pentobarbital group of SDT fatty rats (pentobarbital group, 53.9 ± 4.82 ms vs. MMB group, 45.8 ± 3.2 ms, $p = 0.0046$) (Fig. 4d). The peak time of the ΣOPs was significantly longer in the pentobarbital group of SDT fatty rats (pentobarbital group, 144.7 ± 11.8 ms vs. MMB group, 127.0 ± 6.95 ms, $p = 0.0039$) (Fig. 4e). The peak time of the b-wave was significantly longer in the MMB group of SDT fatty rats (pentobarbital group, 69.2 ± 3.16 ms vs. MMB group, 79.2 ± 3.42 ms, $p = 0.0209$) (Fig. 2d). The ERG b-wave and ERG OP waves also were observed at 0.0001 cd.s/m^2 in the SD rats and SDT fatty rats MMB group (Fig. 5b, d) but not in the pentobarbital groups of SDT rats and SDT fatty rats (Fig. 5a, c).

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Discussion/Conclusion

This is the first report to investigate the effect of anesthetic agents on the ERG in SDT fatty rats. ERGs are performed to detect abnormal retinal function. In diabetic patients, the peak latency time occurs early in the ERG even if there is no change in the ophthalmoscopic examination [17].

Previous animal studies, in which the ERG was measured using Goto-Kakizaki rats and streptozotocin-induced diabetic rats, reported photoreceptor dysfunction and peak time extensions [5, 21]. SDT rats are established approximately 20 weeks after the onset of diabetes and have previously been reported to have reduced ERG amplitudes [10, 22]. SDT fatty rats showed dysfunctional ERGs before histologic changes [10]. The comparison of SDT rats and SD rats showed no significant differences at an early stage; however, 44 weeks after the onset of diabetes, a decreased amplitude of the OP waves and an extended peak time were observed. Prolongation of the peak time in the ERG occurs early in humans. The SDT rat has an extended latency in late diabetes. To evaluate the retinal function, it is important to analyze the amplitude and peak time of the OP waves. The ERG in SDT fatty rats has been reported to extend the peak time at 16 and 24 weeks [13].

The effects of anesthetic agents on the ERG have been reported [23–27]. Several studies have reported changes in the ERG waveforms when pentobarbital sodium was administered [16]. Sugimoto et al. [28] reported that the OP waves were obscured in mice anesthetized with pentobarbital, but there was no significant difference between the a- and b-waves compared to mice anesthetized with ketamine/xylazine. In the current study, OP4 and a-wave amplitudes were significantly attenuated in the pentobarbital group in Jcl:SD rats, whereas OP2 amplitudes were significantly attenuated in the MMB group. In the pentobarbital group of Jcl:SD rats, the peak time was significantly extended except for the b-wave. The amplitudes of OP1 and OP4 of the SDT fatty rat were significantly attenuated in the pentobarbital group, while the amplitudes of the OP2 and b-waves were significantly attenuated in the MMB group. In the pentobarbital group, the peak time was significantly extended except for the b-wave. Care should be taken when interpreting the OP, especially when pentobarbital is used as an anesthetic agent, as it significantly increased the peak time latency. We recommend MMB as the anesthetic agent when measuring ERGs in diabetic model rats.

The OP waves consist of multiple small waves that appear superimposed on the ascending of the b-wave. OP waves are thought to originate from the inner retina, which contains amacrine cells. Several types of amacrine cells, especially the dopaminergic type, are thought to be associated with the OP waves. OP1 and OP2 may reflect the rod system, and OP3 and OP4 may reflect the cone system [29]. Midazolam contained in MMB is a benzodiazepine, and these drugs have been reported to exert a sedative effect by enhancing the action of gamma-aminobutyric acid (GABA) on the $GABA_A$ receptors [30]. Amacrine cells include dopaminergic cells and GABAergic cells [31]. We speculated that $GABA_A$ receptor activation is responsible for the high-amplitude OP waves. However, pentobarbital sodium reduces the OP wave amplitude by affecting the bipolar cells and Müller cells [32]. The results indicate that MMB and pentobarbital have the opposite effect on the OP waves.

Our report has limitations. Since the subject was a diabetic model animal, the cone system was not measured. In the past comparative experiments of anesthetics using normal mice, it was reported that there was no statistical difference in the cone system [15].

In conclusion, our data suggest that ERG OP waves are significantly affected by anesthetics. The same anesthetic should be used when measuring ERG. Alternatively, the anesthetic used should be carefully analyzed when analyzing the data. By using MMB, it is possible to capture small changes in the ERG-OP waves. We recommend MMB, especially for diabetic animal models such as the SDT fatty rat.

Author Contributions

Conceptualization and writing – original draft: Tetsuya Hasegawa. Data curation: Rina Takagi. Formal analysis: Yoshiaki Tanaka. Funding acquisition: Akihiro Kakehashi. Methodology: Toshikatsu Kaburaki. Project administration: Yasushi Kageyama. Supervision: Akihiro Kakehashi, Toshikatsu Kabtaki, and Shin-ichi Muramatsu. Validation: Takeshi Ohta and Masami Shinohara. Visualization: Tomohiko Sasase.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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