JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS
Volume 38, Number 2, 2022
Mary Ann Liebert, Inc.
DOI: 10.1089/jop.2021.0084

Comparisons of α2-Adrenergic Agents, Medetomidine and Xylazine, with Pentobarbital for Anesthesia: Important Pitfalls in Diabetic and Nondiabetic Rats

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Abstract

Purpose: Anesthesia is necessary to conduct rodent electroretinograms (ERGs). We evaluated utility of the α2-agonist medetomidine versus xylazine for ERG studies in nondiabetic and diabetic rats. Pentobarbital was included as a comparator.
Methods: Male Sprague–Dawley rats, with and without streptozotocin (STZ)-induced diabetes, were anesthetized with medetomidine (1 mg/kg), xylazine (10 mg/kg) (both with ketamine 75 mg/kg), or pentobarbital (70 mg/kg). The depth of anesthesia was assessed, and if adequate, scotopic ERGs were recorded. Blood glucose was monitored.
Results: In nondiabetic rats, all three agents induced satisfactory anesthesia, but with differing durations: medetomidine > pentobarbital > xylazine. ERG responses were similar under medetomidine and xylazine, but relatively reduced under pentobarbital. Both α2-agonists (but not pentobarbital) elicited marked hyperglycemia (peak values 316.1 ± 42.6 and 300.3 ± 29.5 mg/dL, respectively), persisting for 12 h. In diabetic rats, elevated blood glucose concentrations were not affected by any of the agents, but the depth of anesthesia under medetomidine and xylazine was inadequate for ERG recording.
Conclusions: In nondiabetic rats, medetomidine and xylazine elicited comparable effects on ERGs that differ from pentobarbital, but both perturbed glucose metabolism, potentially confounding experimental outcomes. In STZ-diabetic rats, neither α2-agent provided adequate anesthesia, while pentobarbital did so. Problems with α2-anesthetic agents, including medetomidine, must be recognized to ensure meaningful interpretation of experimental results.

Keywords: α2 adrenoceptor, anesthesia, diabetes, electroretinogram, hyperglycemia, medetomidine

Introduction

Electroretinogram (ERG) is a common procedure for assessing retinal function and experimental therapeutics in rodent models of disease; typically it is performed under general anesthesia.[1] Alterations in retinal electrophysiological responses are considered some of the earliest signs of diabetic retinopathy.[2,3]

Several anesthetic regimens such as isoflurane, pentobarbital, and the α2-agonist xylazine combined with ketamine are widely employed in animal research,[4] but all influence baseline ERGs and therefore could affect experimental outcomes. Each has advantages and disadvantages. For example, isoflurane induces stable anesthesia in a highly controllable manner, but it requires specialized vaporizer and exhaust equipment, and the nose-cone attachment may

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interfere with ERG electrode placement; it also markedly affects the scotopic threshold response.[5] Pentobarbital has served as a convenient injectable agent for decades, but its respiratory and cardiovascular suppressant effects are potential risks.

Currently, one of the most commonly used agents is xylazine, an analog of clonidine; it is an α2-adrenergic agonist sedative and analgesic.[6] Xylazine, when combined with the dissociative agent ketamine, induces relatively superficial and short-term anesthesia,[7] which can readily be reversed by the α2-adrenergic antagonist atipamezole. However, xylazine/ketamine may be insufficient for more complex experimental procedures that require a longer duration and/or a greater depth of anesthesia.

Medetomidine, a newer α2-adrenergic agonist sedative and analgesic, has ~200-fold higher potency and 10-times more selectivity than xylazine[8] and provides more consistent anesthesia.[9] It has been increasingly used in species ranging from humans (in its active enantiomer form, dexmedetomidine) to rodents. Specifically, its effect on ERGs has been investigated in detail in horses,[10] dogs,[11–15] cats,[16] pigeons,[17] and sea turtles,[18] alone or in combination with ketamine. In those studies, ERG responses were generally comparable or slightly greater in amplitude with medetomidine than xylazine. Recently, its effect on ERGs has been evaluated in mice in a complex regimen: both a- and b-wave responses were larger with the midazolam, medetomidine, and butorphanol tartrate combination than xylazine/ketamine and pentobarbital.[19]

In the rat, one preliminary report compared the fentanyl, medetomidine, and midazolam (FMM) and fentanyl, dexmedetomidine, and midazolam (FDM) combinations versus other anesthetics.[20] Intriguingly, both FMM and FDM were associated with similar a-wave amplitudes but smaller b-wave amplitudes than xylazine/ketamine. There is, however, little information on rat ERGs under medetomidine anesthesia as compared with other anesthetics in relatively simple regimens.

Previous studies have shown that α2-agonists were generally less effective in inducing anesthesia in rodents with streptozotocin (STZ)-induced diabetes.[21,22] It is therefore unclear whether medetomidine, a more potent drug of the class, might be used for ERG evaluation in diabetic retinopathy research. In this study, we compared the anesthetic efficacy of medetomidine/ketamine, xylazine/ketamine, and pentobarbital in rats without and with STZ-induced diabetes. We also assessed blood glucose responses, and when anesthesia was adequate, ERG responses.

Methods

Animals

Male Sprague–Dawley rats weighing 200–400 g were bred and maintained in a controlled environment (12-h light/12-h dark cycle, temperature 25°C, and 40%–60% humidity), with access to food and water *ad libitum*, at the Biological Services Unit of Queen's University Belfast. The anesthesia and ERG procedures were conducted during daytime; blood glucose concentrations were measured repeatedly up to 24 h. All experiments were approved by the Animal Welfare and Ethics Review Board and conducted with a UK Project License under the Animals (Scientific Procedures) Act 1986, in adherence to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Induction of diabetes and anesthesia

Diabetes was induced by STZ (60 mg/kg, i.p.); control rats received the vehicle citrate buffer. Hyperglycemia (blood glucose ≥300 mg/dL) was confirmed in diabetic rats after 2 weeks and was maintained for 15 weeks. Then, rats were randomly assigned to receive one of the following anesthetic regimens: (1) medetomidine (Dormitor, Pfizer, Exton, PA) 1 mg/kg + ketamine (Ketaset, Fort Dodge, IA) 75 mg/kg ($n=6$); (2) xylazine (Rompun, Bayer, Shawnee Mission, KS) 10 mg/kg + ketamine 75 mg/kg ($n=6$); or (3) pentobarbital sodium (Ayrton Saunders, Cheshire, UK) 70 mg/kg ($n=5$). The selection of dosages was based on our initial dose-exploring experiments, in consultation with the veterinarian at Queen's University Belfast, with the goal of maximizing anesthetic efficacy while avoiding anesthesia-related death.

The α2-agonists were administered subcutaneously to avoid undesirable peritoneal irritation due to their acidity, while both ketamine and pentobarbital were given by intraperitoneal injection. Only a single dose of each agent was administered, and there was no additional ''top-up'' dosing.

The depth of anesthesia was assessed by the presence or absence of pedal, tail, and corneal reflexes every 15 min for the first hour, and again at 2 h. Body temperature was maintained using a warming blanket (Kobayashi Healthcare, London, UK), and monitored with a rectal probe during anesthesia. The study was conducted in a nonblinded manner, with the anesthetic agents administered by an experienced animal facility staff and assessment of neural reflexes (i.e., either positive or negative) confirmed by two scientists to minimize experimental bias.

ERGs recording

For evaluation of full-field scotopic ERGs, rats were dark adapted overnight, anesthetized with one of the regimens above, and placed on a warming pad (Kobayashi Healthcare Ltd, London, UK) with the head secured on a stereotaxic device. The pupil was dilated with Minims® 2.5% (w/v) phenylephrine hydrochloride and Minims 1% (w/v) atropine sulfate (both from Chauvin Pharmaceuticals Ltd., Surrey, UK).[23] The cornea was protected with Viscotears (Bausch & Lomb UK Ltd., Surrey, UK) and the contralateral eye covered by a dark eye patch. ERGs were recorded by the color mini-Ganzfeld (LKC Technologies, Gaithersburg, MD) one eye at a time, using gold electrodes placed on the cornea, with the reference and ground electrodes placed at the nasal fornix and tail, respectively.

The stimulus–response curves for a- and b-wave amplitudes were generated from an average of five responses at eight light intensities between 0.008 and 25 cd*s/m^2. The interstimulus intervals were 10 s at 0.008 and 0.025 cd*s/m^2, 15 s at 0.08–0.8 cd*s/m^2, and 30 s at 2.5–25 cd*s/m^2.

After completion of recording from one eye, the animal's posture was adjusted and ERG measures were obtained from the other eye. Only those animals in which ERG responses were successfully recorded from both eyes were included for data

analysis; averaged data from the two eyes were reported. The amplitude of a-waves was measured from the baseline to the negative deflection trough, and that of b-waves was determined from the a-wave trough to the b-wave peak. The time-to-peak (implicit time) for these waves was also calculated.

To quantify oscillatory potentials (OPs), ERGs in response to the 25 cd*s/m^2 light intensity were processed with a 75 Hz low-pass filter. The amplitude and implicit time were analyzed in reference to the published methods.[19] Briefly, the amplitude of OP1 was measured from the baseline to the peak of OP1, and those for OP2, OP3, OP4, and OP5 were determined from the lowest point of the immediately preceding negative wavelet to the respective wavelet peak. The implicit time of each OP was calculated from the time of stimulation to the time of the respective wavelet peak. For those without an identifiable OP5 wavelet, a value of zero was used in calculation. The total OP amplitude or implicit time was expressed as the sum of OP1-5 (ΣOP).

Blood glucose measurements

Blood glucose concentrations were measured repeatedly from the tail vein using a Contour XT glucometer (Bayer, Germany)[24,25] for the first 2 h in diabetic rats and for 24 h in nondiabetic rats following anesthesia induction. Baseline blood glucose measurements were also obtained from one of the above animal groups in the absence of anesthesia.

Data analysis

Data are presented as mean ± standard deviation or percentage. Statistical significance for continuous data (ERGs and blood glucose concentrations) were determined by 2-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test, or 1-way ANOVA followed by Tukey's or Dunnett's *post hoc* test as appropriate. Categorical data (anesthetic efficacies) were analyzed using Chi-square. All data were processed by Prism 8 (GraphPad Software, La Jolla, CA). $P \leq 0.05$ was considered significant.

Results

Efficacy of anesthetic agents on nondiabetic versus diabetic rats

We determined the percentage of animals showing the presence/absence of neural reflexes, as an indication of anesthetic efficacy, over 2 h under anesthesia with the three regimens (Fig. 1). In nondiabetic rats, all three regimens induced satisfactory depth of anesthesia: all animals anesthetized with medetomidine/ketamine and pentobarbital developed complete loss of pedal, tail, and corneal reflexes; under xylazine/ketamine anesthesia, all animals lost pedal reflex, whereas 5 of the 6 animals also lost tail and corneal reflexes. Medetomidine/ketamine induced the longest anesthesia ($\sim$2 h), followed by pentobarbital ($\sim$1 h). Xylazine/ketamine anesthesia was more transient, with the recovery beginning after 30 min.

Anesthetic efficacy under the two α2-agonists, medetomidine and xylazine (both combined with ketamine), was markedly reduced in STZ-induced diabetic animals. Only two of the six diabetic rats (33%) lost pedal, tail, and corneal reflexes under medetomidine/ketamine, and only one of the six (17%) responded to xylazine/ketamine, and then only transiently. All diabetic animals were adequately anesthetized with pentobarbital (Fig. 1).

Effects of anesthetic agents on ERGs

We compared scotopic ERGs in adequately anesthetized nondiabetic rats according to the anesthetic agent employed. Overall, medetomidine and xylazine (both combined with ketamine) showed comparable ERG responses (Figs. 2 and 3). The a-wave amplitudes were identical between the two. Although not statistically significant, b-wave amplitudes for medetomidine were 17%–42% larger than those for xylazine across the light intensities tested. The a-wave implicit time was similar between the two agents, but that for the b-wave was modestly lower with medetomidine than xylazine ($P = 0.049$).

In contrast, the rats under pentobarbital anesthesia exhibited lesser ERG responses (Figs. 2 and 3). Compared with medetomidine and xylazine (both with ketamine), a-wave amplitudes were reduced over light intensity ranges, 0.8–25 cd*s/m^2 and 0.25–25 cd*s/m^2, respectively (both $P \leq 0.05$). The b-wave amplitude was also lower for pentobarbital versus medetomidine at 0.008 cd*s/m^2 and over the intensity range 0.25–25 cd*s/m^2 ($P \leq 0.05$). Pentobarbital anesthesia showed longer a-wave implicit time ($P \leq 0.05$) and shorter b-wave implicit time ($P \leq 0.001$) than either medetomidine or xylazine.

We measured OPs in a subset of the nondiabetic animals. Figure 4 illustrates the filtered OPs from nondiabetic rats anesthetized with the three regimens. Overall, the magnitude of waveforms was in the following order: medetomidine > xylazine > pentobarbital. OP5 was not noticeable under pentobarbital anesthesia. Of the wavelets analyzed, the amplitude was higher for medetomidine/ketamine versus pentobarbital at OP2-5 ($P \leq 0.01$), and for xylazine/ketamine versus pentobarbital at OP3 ($P \leq 0.01$) and OP4-5 ($P \leq 0.05$). When the sums of OP1-5 amplitudes were analyzed, there were significant differences between all three agents: $P \leq 0.0001$ for medetomidine/ketamine versus pentobarbital, $P \leq 0.001$ for xylazine/ketamine versus pentobarbital, and $P \leq 0.05$ for medetomidine/ketamine versus xylazine/ketamine. Additionally, significant differences in implicit times were found for medetomidine/ketamine versus pentobarbital (OP1: $P \leq 0.01$, OP5: $P \leq 0.0001$, and sum of OP1-5: $P \leq 0.001$) and for xylazine/ketamine versus pentobarbital (OP1: $P \leq 0.05$, OP5: $P \leq 0.0001$, and sum of OP1-5: $P \leq 0.01$).

Due to the inadequacy of anesthesia induced by medetomidine or xylazine in diabetic rats, we were unable to obtain reliable ERG data for analysis. However, ERGs were readily measurable in diabetic rats under pentobarbital anesthesia: both a- and b-wave responses were reduced in comparison with nondiabetic rats at the higher light intensity range (Supplementary Fig. S1). This difference may be attributable to the development of diabetic retinopathy, but theoretically could also involve an interaction between the anesthetic agent and diabetes.

Effects of anesthetic agents on blood glucose

In nondiabetic rats, both medetomidine and xylazine elicited rapid and severe hyperglycemia sustained throughout

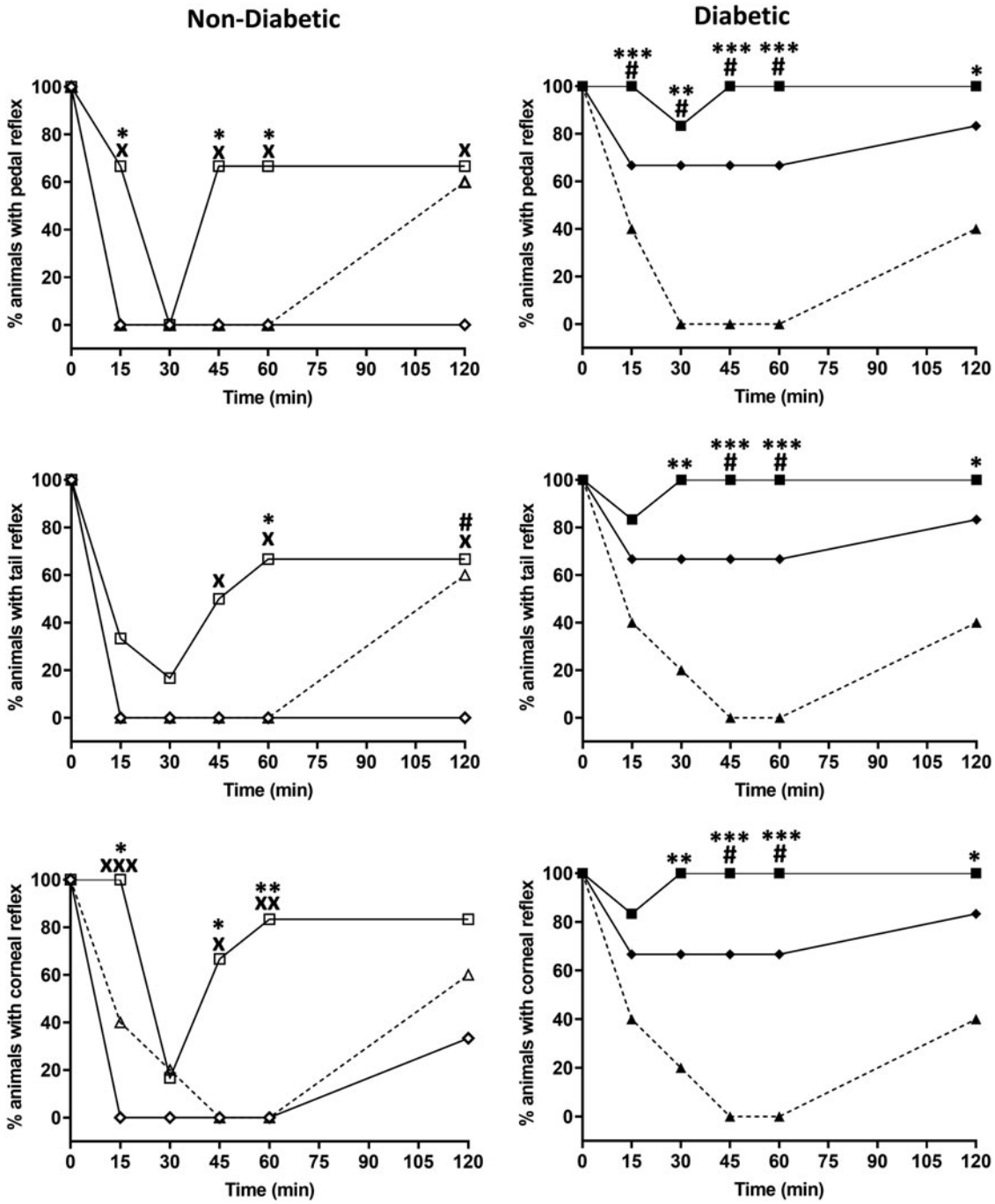

FIG. 1. Time course anesthetic effects of α2-adrenoceptor agonists and pentobarbital in nondiabetic and STZ-induced diabetic rats. Animals were administered with medetomidine 1 mg/kg ($n = 6$), xylazine 10 mg/kg ($n = 6$; both combined with ketamine 75 mg/kg), or pentobarbital 70 mg/kg ($n = 5$). Anesthetic depth was assessed by pedal (*upper*), tail (*middle*), and corneal reflexes (*lower*) over 2 h. Data were expressed as % animals showing a positive neural reflex at each time point. *Diamond*: medetomidine/ketamine; *square*: xylazine/ketamine; *triangle, perforated line*: pentobarbital. #Medetomidine/ketamine versus pentobarbital; *xylazine/ketamine versus pentobarbital; xmedetomidine/ketamine versus xylazine/ketamine. $^{\#/*/x}P \leq 0.05$, $^{\#\#/**/xx}P \leq 0.01$, $^{\#\#\#/***/xxx}P \leq 0.001$. STZ, streptozotocin.

and beyond the duration of the ERG procedure, with peak blood glucose levels reaching 316.1 ± 42.6 mg/dL at 4 h for medetomidine and 300.3 ± 29.5 mg/dL at 1 h for xylazine (Fig. 5). Hyperglycemia gradually recovered by ~12 h, and there then ensued a period of hypoglycemia from 18 h until ~24 h in medetomidine-anesthetized rats ($P < 0.05$ vs. conscious rats at 19–21 h). In contrast, nondiabetic rats receiving pentobarbital maintained normal blood glucose during and after the experiment (peak 120.7 ± 9.6 mg/dL).

In diabetic rats, glucose concentrations were measured at multiple time points over 1 h after administration of the

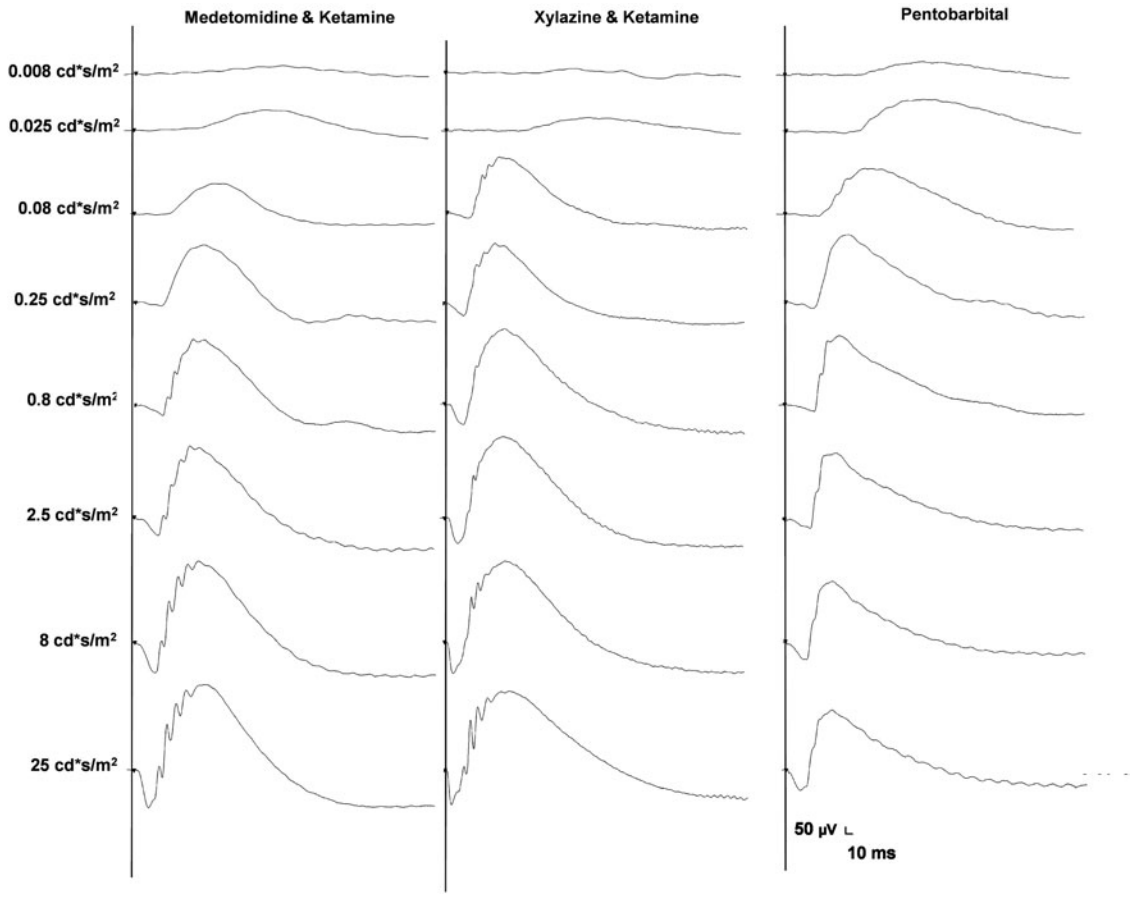

FIG. 2. Representative full-field scotopic ERG recordings from nondiabetic rats. Animals were dark adapted overnight, and then anesthetized with medetomidine (1 mg/kg), xylazine (10 mg/kg; both combined with ketamine 75 mg/kg), or pentobarbital (70 mg/kg). The pupil was dilated with topical 2.5% (w/v) phenylephrine and 1% (w/v) atropine. ERGs were recorded by a color miniature Ganzfeld, using gold electrodes placed on the cornea and the reference and ground electrodes at the nasal fornix and tail, respectively. Waveforms between light intensities of 0.008–25 cd*s/m^2 are shown. Scale bar indicates 50 µV and 10 ms. ERG, electroretinogram.

three anesthetic regimens and again at 2 h. None of the agents showed any significant effect on the already elevated glucose concentrations (Supplementary Fig. S2).

Discussion

Anesthetic agents can modulate retinal activity, potentially through direct (i.e., neuronal) and indirect (e.g., metabolic and homeostatic) processes. They not only influence basal ERG responses, but may also interfere with experimental outcomes.[26] To our knowledge, this report is the first to compare medetomidine/ketamine, xylazine/ketamine, and pentobarbital as agents for rat ERG studies. We studied both diabetic and nondiabetic animals, encountering concerns with anesthetic efficacy in the former, and with prolonged and severe hyperglycemia in the latter.

In these studies, each of the α2-agents was combined with a same dosage of ketamine to permit direct comparisons. In nondiabetic rats anesthetized with medetomidine, a-wave responses (reflecting photoreceptor function) were nearly identical to those with xylazine. The b-wave amplitudes (from second-order processes, including bipolar and Müller cells) were higher overall with medetomidine than xylazine, although the difference did not reach statistical significance. Compared with either α2-agent pentobarbital was associated with subdued a- and b-waves. Similarly, OPs showed significantly reduced amplitudes and shortened waveforms with pentobarbital versus the α2-agents, especially medetomidine, consistent with their known sensitivity to γ-aminobutyric acid (GABA) antagonism.[27]

Thus, our data suggest that α2-agents, particularly medetomidine, are superior to pentobarbital for ERG studies. Importantly, however, this advantage is countered and may be outweighed by their undesirable hyperglycemic effects in nondiabetic rats and by their lack of anesthetic efficacy in diabetic animals. For STZ-diabetic rats, pentobarbital may be an option, considering its much superior anesthetic efficacy.

α2-Adrenoceptors are widely distributed in both the central nervous system and the periphery, mediating a myriad of global effects, including sedation, nociception, muscle relaxation, thermoregulation, sympathetic output, hypotension, and vasoconstriction in certain blood vessels. They are also expressed in the retina.[28–31] Woldemussie et al.[32] reported that all three α2-adrenoceptor subtypes are present and differentially localized in the rat retina: α2A is in the somata of ganglion cell and inner nuclear layers, α2B is in the dendrites and axons of most neurons and glia across all retinal layers, and α2C is restricted to the somata and inner segments of photoreceptors.

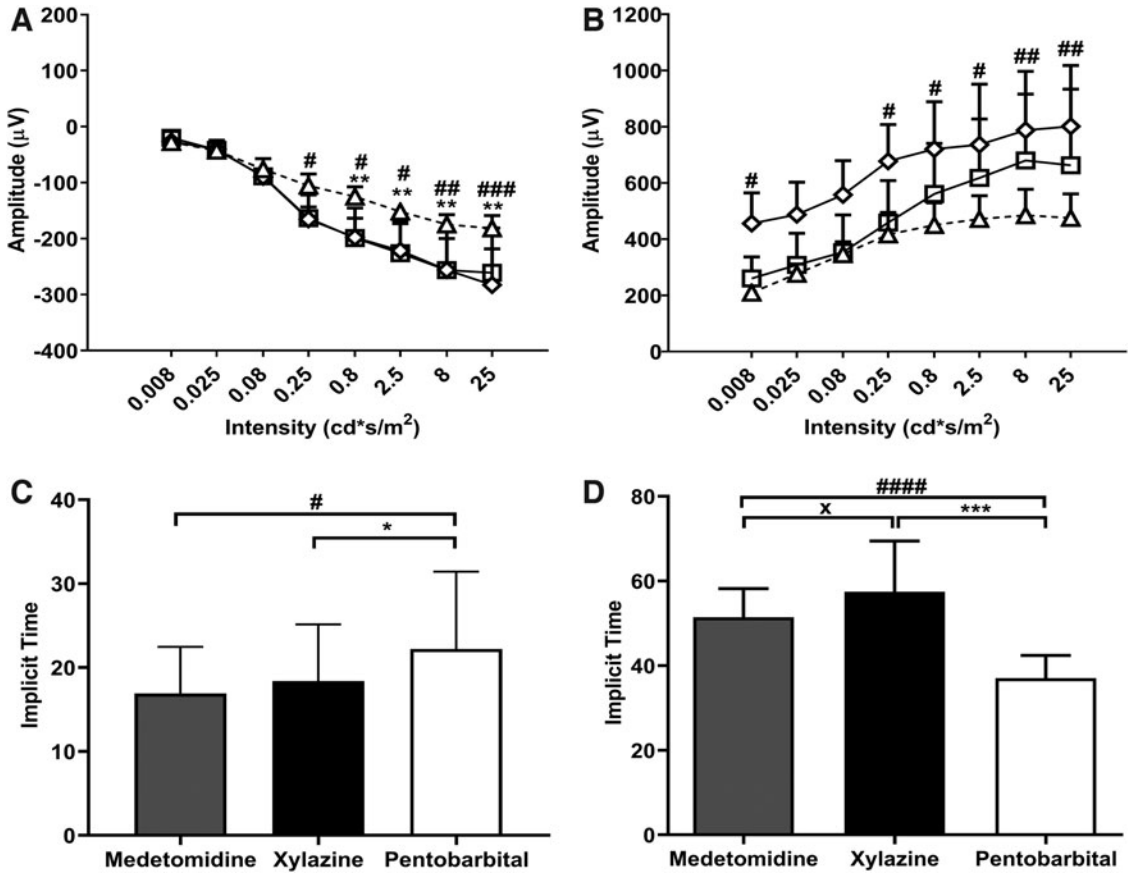

FIG. 3. A- and b-wave ERG responses from nondiabetic rats. ERGs were recorded as in Figure 2. Data (a-waves in panels **A and C**; b-waves in panels **B and D)** are summarized as light intensity–response curves for the amplitude, and as average responses across the light intensity range for the implicit time. Mean ± SD, $n = 4$–5 per anesthetic regimen. *Diamond*: medetomidine/ketamine; *square*: xylazine/ketamine; *triangle, perforated line*: pentobarbital. #Medetomidine/ketamine versus pentobarbital; *xylazine/ketamine versus pentobarbital; xmedetomidine/ketamine versus xylazine/ketamine. $^{\#/*/x}P \leq 0.05$, $^{\#\#/**/xx}P \leq 0.01$, $^{\#\#\#/***/xxx}P \leq 0.001$, $^{\#\#\#\#/****/xxxx}P \leq 0.0001$. SD, standard deviation.

Since both medetomidine and xylazine activate all α2-adrenoceptor subtypes,[33] they may exert a broad range of effects. Functionally, α2-agonists, including medetomidine have been shown to suppress calcium signaling in the inner plexiform layer where retinal ganglion cells communicate with presynaptic bipolar and amacrine cells[34,35] they are also thought to be neuroprotective.[30,36]

Besides direct retinal neuronal actions, other ocular effects of α2-agonism, including centrally mediated mydriasis,[37] decreased intraocular pressure,[38] and altered retinal blood flow,[39] may also contribute to ERG responses (although in general, the pupils are dilated by topical mydriatics). Anesthesia-induced acute corneal[40,41] and lens opacification[42,43] has been reported, which may interfere with the light path, although in the current study we did not observe such changes and ERG responses under the α2-agents were robust, likely because the cornea was well moisturized. Moreover, Sprague–Dawley rats are relatively resistant to corneal lesions.[41]

Compared with xylazine, medetomidine has 200-fold greater potency at α2-adrenoceptors, and is more selective for α2- over α1-adrenoceptors.[8] It is more lipophilic than xylazine, although plasma elimination half-lives of the two agents are comparable.[44,45] The dosage for medetomidine used in this study was one tenth that of xylazine. The attributes of medetomidine are consistent with our observation that it elicited a modestly higher degree of ERG responses than xylazine.

Our findings in nondiabetic rats are consistent with those in larger mammalian species, such as horses,[10] dogs,[12] and cats.[16] In each, ERG responses were similar or slightly greater with medetomidine than xylazine, whether administered solely or in combination with ketamine. However, drug dosages varied across the studies and species: for instance, in the feline study,[16] the dose for medetomidine (5 μg/kg) was only 1/200 of that for xylazine (1 mg/kg).

Recently in mice, the effect on ERGs of anesthetic combinations, including medetomidine has been compared with xylazine/ketamine and pentobarbital.[19] In rats, medetomidine anesthesia has been used for ERG recording,[46–48] yet only one study compared combinations that included medetomidine with those including xylazine.[20] In that study, use of medetomidine was associated with similar a-wave amplitudes but smaller b-wave amplitudes than xylazine, but in view of multiple variables, it is difficult to attribute effects specifically to either agent.

Importantly, both medetomidine and xylazine significantly raised blood glucose of healthy nondiabetic rats to levels characteristic of diabetes. These effects were sustained for periods commensurate with pharmacologic

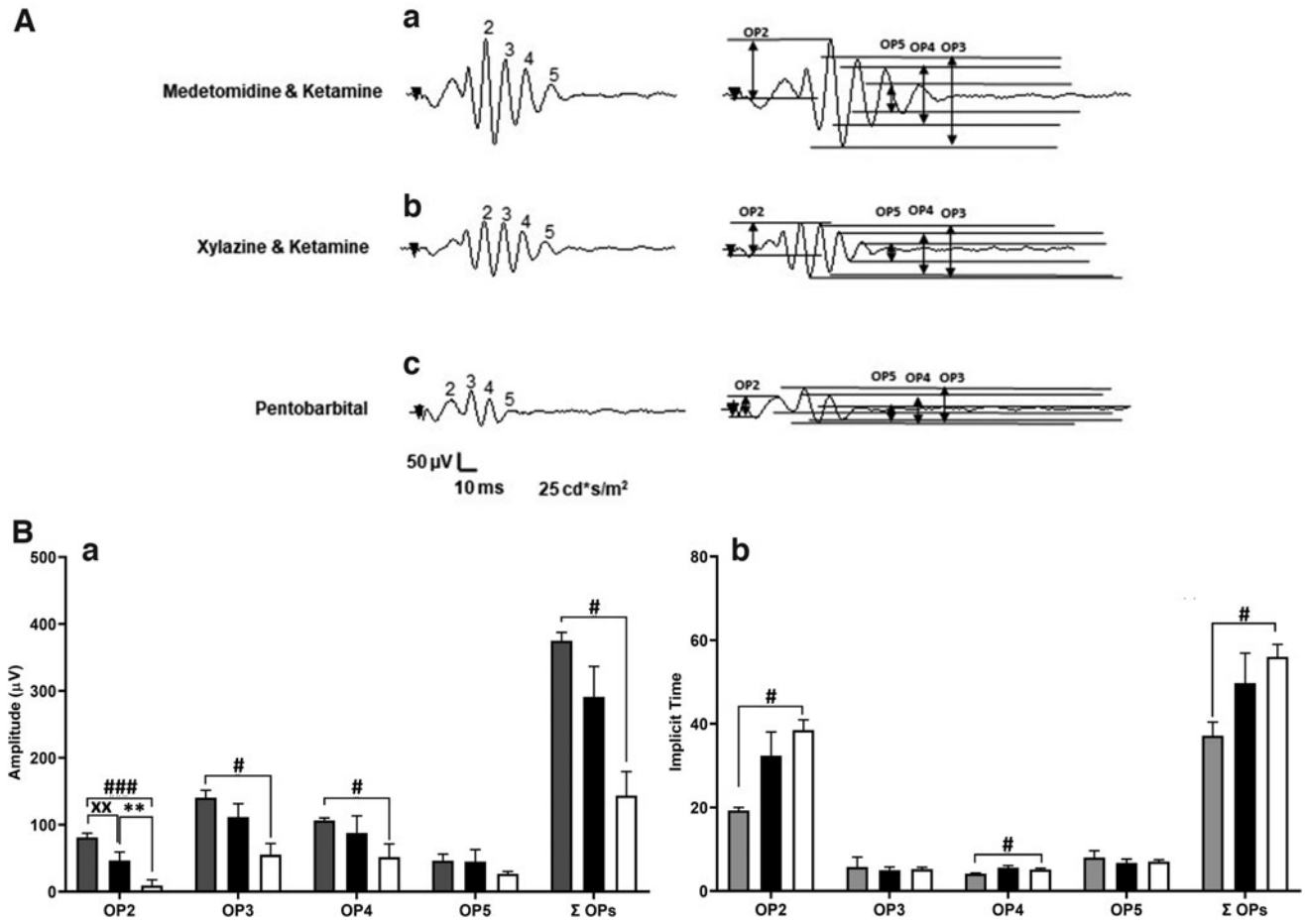

FIG. 4. Filtered scotopic OPs from nondiabetic rats. **(A)** Representative OPs under anesthesia with (a) medetomidine/-ketamine, (b) xylazine/ketamine, and (c) pentobarbital. ERGs were recorded in response to the light intensity of 25 cd*s/m^2, with a 75 Hz low-pass filter. Calculations for the amplitude are illustrated. **(B)** Amplitudes and implicit times of individual OPs and sum of OP1-5 (ΣOP), expressed as mean ± SD, $n = 3$ per anesthetic regimen. #Medetomidine/ketamine versus pentobarbital; *xylazine/ketamine versus pentobarbital; xmedetomidine/ketamine versus xylazine/ketamine. #/*/x$P \le 0.05$, ##/**/xx$P \le 0.01$, ###/***/xxx$P \le 0.001$. OPs, oscillatory potentials.

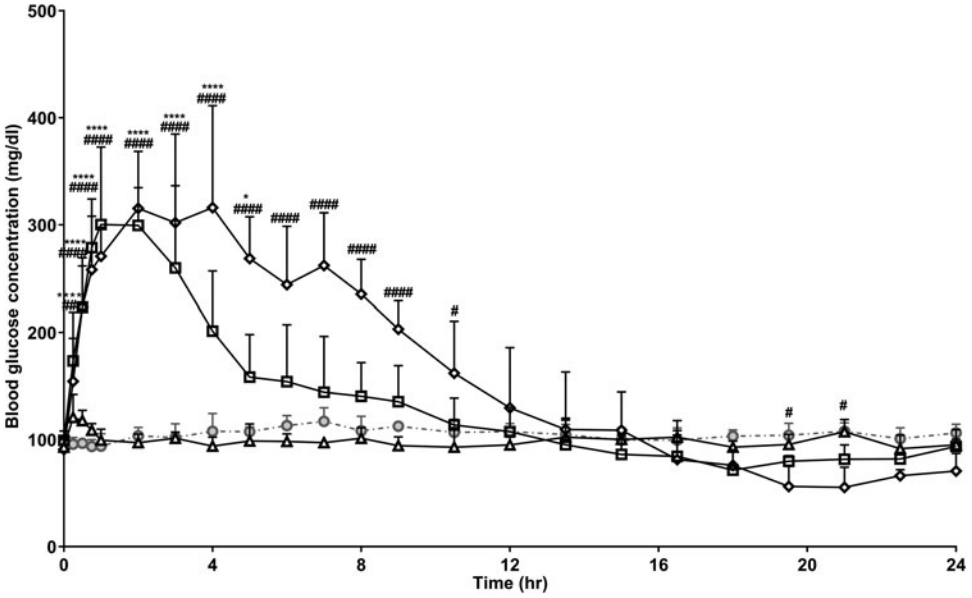

FIG. 5. Blood glucose concentration changes by α2-adrenoceptor agonists and pentobarbital in nondiabetic rats. Animals received medetomidine 1 mg/kg ($n = 6$), xylazine 10 mg/kg ($n = 6$; both combined with ketamine 75 mg/kg), or pentobarbital 70 mg/kg ($n = 5$), versus without anesthesia ($n = 6$). Blood glucose concentrations were measured for 24 h after administration and expressed as mean ± SD. #Medetomidine/ketamine versus nonanesthetized; *xylazine/ketamine versus anesthetized. #/*$P \le 0.05$, ##/**$P \le 0.01$, ###/***$P \le 0.001$.

potencies, that is, ~12 h, covering the duration of most common experimental procedures. These data are consistent with earlier reports,[49–53] but we are concerned that this problem is not widely appreciated: it is almost never mentioned in the retinopathy literature, and in our interactions with colleagues, we found a universal lack of awareness.

Hyperglycemia may be caused by reduced insulin release mediated by α2-adrenoceptors on pancreatic β-cells. The subsequent phase of relative hypoglycemia from 18 to 24 h likely reflects a compensatory insulin release after the drug action has worn off. Intraprocedure hyperglycemia could theoretically complicate experimental outcomes: ERGs are sensitive to acute glucose changes in both nondiabetic and diabetic conditions,[54,55] although one study reported no effect in nondiabetic rats.[56] Acute hyperglycemia, and subsequent hypoglycemia, may cause changes in tissue osmolality, hemodynamics, rheology, and metabolism. Transient high glucose in cultured aortic endothelial cells (for 16 h), and in nondiabetic mice (for 6 h), has been reported to induce epigenetic changes and increase p65 gene expression lasting for over 6 days despite glycemic normalization.[57]

In contrast to nondiabetic rats, in STZ-diabetic rats, we found that neither medetomidine nor xylazine was an effective anesthetic agent. This lack of efficacy in diabetic rodents was first noted over 30 years ago,[21,22] but α2-agents remain in common use in diabetic rodent research today. Again, we have found a widespread lack of awareness of the issue. A common practice to circumvent inadequate anesthesia is to administer ''top-up'' doses. In our own experience, this did not improve outcomes, but instead led to frequent deaths. Higher doses may not only compromise animal welfare, but also confound ERG readings and other endpoints, and potentially invalidate the purported case–control principle.

Possible explanations for poor efficacy in diabetic animals include faster drug clearance (e.g., through increased urination)[21] and altered pharmacodynamics: diabetes may modify α2-adrenoceptor function, possibly by reducing receptor density or affinity, or by interfering with signaling. This dysfunction could be reversed by insulin supplementation, suggesting a role of insulin deficiency.[22,58,59]

We included pentobarbital, a classical $GABA_A$ receptor agonist, as a reference agent. Pentobarbital provided effective anesthesia in both diabetic and nondiabetic rats, without disturbing glucose metabolism. This drug, despite its narrow safety margin, may be an alternative for diabetic retinopathy research. However, its depressant effect on the baseline ERG waves, including OPs, should be considered in experimental design and data interpretation. Recent evidence shows that some of the triple fentanyl regimens may achieve surgical anesthesia without large disturbances in hemodynamic, metabolic, and inflammatory profiles in mice.[60] However, in our pilot study, fentanyl–fluanisone–midazolam was unsuccessful in inducing anesthesia in STZ-diabetic rats (data not shown). Complex regimens may also introduce different experimental variables.

In humans, ERGs are typically measured in the conscious setting, which is preferable to observe unaltered electrophysiological responses. New technologies for conscious ERG studies in various animal models have been developed,[61–64] but it is likely that anesthesia will continue to be used for most rodent studies. While we could not compare data between conscious and unconscious rats, it appears that α2-agonists combined with ketamine minimize the depressant effects on ERGs in rodents, and thus may be superior to other agents in this respect.[63–66] Xylazine/ketamine may actually enhance ERG a- and b-wave amplitudes in rats,[63] while having little effect in mice.[64,67] However, medetomidine alone or dexmedetomidine combined with butorphanol dampened ERG responses in the dog,[11,15] suggesting likely species differences.

Our study has limitations. First, the group sizes are relatively small, especially for the OP experiments, which were introduced part-way through the study. Nevertheless, the OP findings were consistent with changes in a- and b-wave amplitudes, and the large differences in anesthetic efficacy and blood glucose concentrations also provide confidence in the key findings. Second, we investigated STZ-diabetic rat model only. It is unclear whether type 2 diabetes models exhibit similar responses, although one study reported reduced hypothalamic α2A-adrenoceptors in an ob/ob mouse model of metabolic syndrome.[68] It is also unclear whether anesthetic efficacy of α2-agonists is reduced in diabetic humans; circumstantial evidence suggests a likely alteration of α2-adrenoceptor density or function in some patients with diabetes,[69,70] but unlike STZ-diabetic rodents, patients with type 1 diabetes are typically treated with insulin.

Görges et al.[71] recently investigated the effect of dexmedetomidine (0.25–0.75 μg/kg) on blood glucose in nondiabetic children under anesthesia, finding that it caused a dose-dependent but mild elevation of blood glucose at 15 min but not at 30 min. These dosages were lower than those used in the rat, but a case report of a child who accidentally received a 60× dexmedetomidine overdose found significant hypoglycemia but not hyperglycemia.[72] Third, the study was not blinded, although assessment of neural reflexes was verified by two assessors to reduce experimental bias. Last, the inability to achieve adequate sedation in diabetic rats using the α2-agents limited our ability to evaluate their effects on ERGs.

In summary, we found that in nondiabetic rats, medetomidine elicited modestly higher ERG responses than xylazine when administered in conjunction with ketamine. The ERG waves were larger with α2-agents than with pentobarbital. Medetomidine/ketamine may be used for procedures that require an extended anesthesia duration. Importantly, the hyperglycemia associated with the α2-agents must be appreciated and should be factored into experimental design. Additionally, neither medetomidine nor xylazine induced adequate anesthesia in STZ-diabetic rats, and pentobarbital may be an alternative. Greater appreciation of these properties of anesthetic agents is essential for the rational design of meaningful experiments to investigate retinal disorders and therapeutics in rat models with and without diabetes, and to minimize animal suffering.

Acknowledgments

The authors are grateful to Lorraine Hanna (Biological Services Unit, Queen's University Belfast) for her skilled assistance in the animal studies, and to Dr. Jie Fan (Department of Ophthalmology, Medical University of South Carolina) for providing insightful comments to the article.

Authors' Contributions

J.Y.Y., T.J.L., and A.R.C. contributed to the conception and design of the study, data interpretation, and article writing. A.R.C., M.B.H., D.F., and L.H. conducted experiments

and collected data. D.P.B. contributed to data interpretation and article writing. All authors approved the final version of article. T.J.L. and J.Y.Y. are the guarantors of this work.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This work was funded by a Queen's University Belfast PhD scholarship to A.R.C., LINJO fund to T.J.L., and internal support to J.Y.Y. from the Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast and the Department of Medicine, Medical University of South Carolina. D.P.B. is supported by UKRI NC3Rs, MRC, and Invest Northern Ireland. The sponsor or funding organization had no role in the design or conduct of this research.

Supplementary Material

Supplementary Figure S1
Supplementary Figure S2

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Received: September 7, 2021
Accepted: November 1, 2021

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