Retinal Function Loss after Monocarboxylate Transport Inhibition

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PURPOSE. To test the proposal that inhibiting monocarboxylate transport in the rat retina results in altered retinal function measured using the electroretinogram (ERG) and to evaluate the efficacy of exogenous metabolic substrates to restore any functional deficit.

METHODS. Full-field white-flash ERGs were measured after monocarboxylate transport inhibition with intravitreal injection of α-cyano-4-hydroxycinnamic acid (4-CIN, 10 mM), and functional recovery was assessed after the introduction of various exogenous metabolic substrates (10 mM): lactate, pyruvate, α-ketoglutarate, alanine, succinate, and glutamine. The efficacy of glutamine as a metabolic substrate was also considered in the presence of phosphate-activated glutaminase inhibition (6-diazo-5-oxo-norleucin, 10 mM) or aminotransferase inhibition (aminooxyacetic acid, 10 mM). Pyruvate and alanine recovery was also assessed after aminooxyacetic acid application.

RESULTS. 4-CIN application resulted in an increased phototransduction amplitude but a mild reduction of gain. A greater reduction of postreceptor b-wave and oscillatory potential amplitudes (80%) was observed, along with delayed implicit times (35 ms). Partial recovery of b-wave amplitudes was achieved with exogenous lactate (24%), pyruvate (27%), α-ketoglutarate (27%), alanine (25%), and succinate (26%), whereas glutamine provided 62% recovery. However, none of the substrates improved phototransduction gain. Both 6-diazo-5-oxo-norleucin and aminooxyacetic acid completely suppressed the glutamine-induced b-wave recovery. Aminooxyacetic acid also abolished the b-wave recovery from 4-CIN afforded by pyruvate and alanine.

CONCLUSIONS. The greater loss of the b-wave and oscillatory potentials may reflect preferential routing of amino acid carbon skeletons to oxidative metabolic pathways, which in turn reduces glutamate availability for neurotransmission between photoreceptors and ON-bipolar cells. The reduction in log S provides evidence that inhibition of monocarboxylate transport produced some metabolic dysfunction in the rat. (Invest Ophthalmol Vis Sci. 2004;45:584–593) DOI:10.1167/iovs.03-0695

Monocarboxylates are ubiquitous short chain carbon molecules, with a single carboxyl group involved in numerous biological actions. In neuronal systems, key monocarboxylates include pyruvate, α-ketoglutarate, lactate, and ketone bodies (hydroxybutyrate, acetoacetate, acetone), all of which are involved in energy-producing pathways, as summarized in Figure 1. More specifically, the decarboxylation of pyruvate (through pyruvate carboxylase [PC]) is the critical link between glycolysis and the tricarboxylic acid (TCA) cycle,1 whereas α-ketoglutarate is both an important TCA cycle intermediate and a precursor for glutamate production.2 More recently, lactate has been shown to be a useful metabolic substrate during exercise,3,4 hypoglycemia,5–7 and hypoxia.8

Consistent with the intricate role of monocarboxylates in retinal metabolism, multiple isoforms of monocarboxylate transporters (MCTs)9 have been found in the retina. The retinal pigment epithelium expresses MCT1 and MCT3 on its apical and basolateral membranes, respectively.10–14 MCT1 is also expressed in Müller cells, photoreceptor inner segments, and the inner blood–retinal barrier.15,16 In accord with impaired glutamate neurotransmission, 4-CIN decreases the levels of glutamine, a glutamate precursor, whereas the glutamate metabolite aspartate is increased.17 Such a change in amino acid levels is consistent with a shift in the equilibrium of glutamate metabolic pathways, as has been reported secondary to retinal metabolic challenge.18–20 In accord with impaired glutamate neurotransmission, 4-CIN causes a loss of glutamate-mediated excitatory postsynaptic potentials in hippocampal slices.20–22 Hence, inhibiting monocarboxylate transport MCT at both the cellular and mitochondrial membranes using 4-CIN5 (Fig. 1B, filled circles) should impair retinal function, especially neurotransmission. However, it is unclear whether such a neurotransmission deficit occurs secondary to glutamate oxidation for metabolism.

The retina is a useful tissue in which to consider the role of monocarboxylates, as it has a particularly high metabolic demand.24–25 The photoreceptor response to light (a-wave)26,27 can be easily recorded with the electroretinogram (ERG), and this component, known as the P3, can be described relative to well-known phototransduction biochemical cascades.28–30 The subsequent positive b-wave is generated after glutamate-dependent synaptic activity on depolarizing bipolar cells.31–33 In addition, the wavelets on the ascending edge of the b-wave, termed the oscillatory potentials (OPs), are thought to reflect amacrine cell–mediated responses and therefore can provide information regarding inner retinal function.34–36 Hence, we used the in vivo ERG for simultaneous assessment of photoreceptor and postreceptor function after inhibition of MCT by 4-CIN. We also considered the reversibility of the MCT blockade by systematically introducing substrates, which were chosen to probe the aminotransferase reactions involving glutamate. In addition, we tested the hypothesis that the glutamate–glu-
amine cycle contributes carbon substrates to oxidative pathways under metabolic stress (induced by 4-CIN), by simultaneously supplying exogenous glutamine and inhibiting enzymes involved in glutamate metabolism.

**MATERIALS AND METHODS**

**General Procedures**

All experimental protocols in this study were approved by our institutional ethics committee and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Electroretinograms were recorded from the left eye of five adult Long-Evans rats (aged 9 weeks) for each treatment. Animals were maintained in a 12 hour light–dark (40–130 lux, on at 8 AM) environment with normal rat chow and water available ad libitum. Dark-adapted (>12 hours) animals were anesthetized under dim red light ($\lambda_{max} = 650$ nm) with a mixture of ketamine and xylazine (60:5 mg/kg; Troy Laboratories, Smithfield, New South Wales, Australia). Mydriasis ($\lambda_{max} = 4$ mm) was achieved with tropicamide (Mydriacyl 0.5%, Alcon Laboratories, Frenchs Forest, New South Wales, Australia) and corneal anesthesia with proxymetacaine (Opththic 0.5%; Allergan, Frenchs Forest, New South Wales, Australia).

**Electroretinography**

Flash ERGs (white) were recorded with silver-silver chloride (Ag-AgCl) electrodes. The corneal active and mouth inactive electrodes were referenced to a stainless-steel ground (Medelec, Richmond, Victoria Australia) inserted in the tail. After electrode insertion, a further 10 minutes of dark adaptation was allowed before signals were collected from a single flash with a minimum interstimulus interval of 2 minutes. Responses were amplified (gain ×1000; −3 dB at 0.1 and 3000 Hz; model P55 Grass Telefactor Inc., West Warwick, RI) and digitized at 2 kHz. A commercial photographic flash unit (285V; Vivitar Photographics, Newbury Park, CA) was delivered through a Ganzfeld sphere. Flash exposure was calculated as previously described,35 and yielded an unfiltered photopic exposure of 3.5 log cd·s/m², which was attenuated using a calibrated neutral-density filter (1.0 ND; Kodak Wratten, Eastman Kodak Co., Rochester, NY). This luminance energy (2.5 log

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*FIGURE 1.* Schematic of several MCTs and retinal metabolic pathways. (A) Glycolytic breakdown of glucose provides the MCT pyruvate (pyr) for oxidative metabolism through the TCA cycle. The monocarboxylate lactate (lac), may be shunted by MCTs from glial cells and neurons in a scheme termed the lactate shuttle. Recycling of glutamate (glu) to glutamine (gln) between glial cells and neurons is commonly known as the glutamate–glutamine cycle. (B) 4-CIN inhibits MCTs at the cell membrane and the mitochondrial pyruvate carrier. This action is likely to affect substrate availability for glutamate and GABA production and metabolism, including GDH and the aminotransferases (AAT, ALAT, and GABA-T). α-KG, α-ketoglutarate; A-CoA, acetyl-coA; ala, alanine; asp, aspartate; GS, glutamine synthetase; LDH, lactate dehydrogenase; oxal, oxaloacetate; PAG, phosphate activated glutaminase; PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; SSAD, succinate semialdehyde dehydrogenase.
cd s/m^2 was chosen because it elicits both a saturated a-wave, a prominent b-wave, and well formed OPs but is still dim enough to ensure that the time required to recover adequate dark adaptation is reasonable (~2 minutes) during serial ERG measurements.

**ERG Protocol**

Four baseline ERG signals were collected with an interstimulus interval of 2 minutes, and data for each animal were normalized to their respective average parameters derived from these four recordings, to minimize interanimal variability. This approach provided an excellent signal-to-noise ratio in our experimental preparations. After baseline ERGs, pharmacological treatments were applied (as described later) and single signals were collected at 2-minute intervals for 40 minutes. In some cases, exogenous substrates and/or enzyme inhibitors were injected into the vitreous chamber at 40 minutes after treatment. This procedure did not exceed 2 minutes, and a minimum of 3 minutes readaptation was afforded before the next signal was acquired. During the interval between 40 minutes and 3 hours, the interstimulus interval was lengthened to 5 minutes. A single intensity recording protocol was adopted to maintain constant adaptation of the retina, as adaptation state is known to change the metabolic demands.

**ERG Analysis**

Conventional analysis of the photoreceptor a-wave in amplitude and implicit time may be contaminated by changes in the postreceptoral response. We, therefore, describe the phototransduction response using the phototransduction model of Hood and Birch as given in the equation

\[ P(t; t) = RmP \left[ 1 - e^{-t/(t_i)} \right] \text{ for } t > t_i. \]

The P3 is the summed rod photocurrent as a function of stimulus exposure, \( t \) (in cd s/m^2) and time \( t \) (in seconds), and \( RmP \) (in microvolts) is its saturated amplitude. Phototransduction gain \((S, m^2 \cdot cd^{-1} \cdot s^{-1})\) is scaled by \( t \), whereas \( t_i \) (in seconds) is a delay that includes biophysical and other recording latencies. This model was fitted to the raw data up to the first minimum of each a-wave. Optimization was achieved by minimizing the sum-of-square error term with the solver module of a spreadsheet (Excel; Microsoft Corp., Redmond, WA). As per convention, \( t_i \) was fixed to 2.75 ms, whereas we chose to float both \( RmP \) and \( S \) to reflect the dependence of the dark current on retinal metabolism. More complex formulations for phototransduction, which included corrections for membrane capacitance, were not used, as the effect of metabolic impairment on capacitance is unclear. In any case, the equation provides an excellent fit to our data (see Fig. 2). Postreceptoral amplitudes were calculated from the trough of the P3 to the peak b-wave amplitude. The b-wave implicit times were taken from stimulus onset to the peak. The OP amplitude was measured after raw data were band-pass filtered (~5 dB at 70 and 280 Hz) by summing the root mean square (RMS) over the entire OP complex. An OP amplitude (RMS) of more than 20 \( \mu \)V was considered greater than the background noise, which was determined over a 50-ms epoch from noise traces. The implicit time of the OPs was taken as the peak time for the largest oscillation. Extracted parameters were normalized to their pretreatment baseline values and expressed as a mean (± SEM) for each treatment group (\( n = 5 \)), as a function of time elapsed since treatment.

**Statistics**

Statistical comparisons were made using analysis-of-variance (ANOVA; Prism, ver. 3.02; GraphPad Software Inc., San Diego, CA). As our group size was small we used an adjusted \( \alpha \) of 0.01 to protect against type two errors. ANOVA results are quoted with their probabilities, and when nonsignificant results were found, the power of the performed test is shown in parenthesis.

**Pharmacological Agents**

Intravitreal injections have the advantage of direct delivery of the pharmacological agents to the retina, thereby bypassing the blood-retinal barrier. An aliquot of 2 to 3 \( \mu \)L of the pharmacological agent or vehicle was delivered into the vitreous chamber through a plastic-coated 30-gauge needle with 1.5 mm of the tip exposed. Dureau et al. have shown that small injected volumes (1-5 \( \mu \)L) provide good reproducibility and minimize any loss of solution. The needle was inserted 2 mm behind the limbus in the superior retina at a 45° angle to avoid contact with the lens capsule. Data were excluded if opacification of the lens was detected (~1/20 eyes). All agents were diluted in phosphate-buffered saline and equalized approximately to pH 7.4, using 1 M hydrochloric acid and 1 M sodium hydroxide (BDH Chemicals, Kilnsyth, Victoria, Australia). The concentrations quoted represent the adjusted vitreous chamber volume of 40 \( \mu \)L for the rat. This volume is based on previous measurements and our own calculations determined from the oculur dimensions provided by Hughes.

**MCT Inhibition**

We adopted a calculated final vitreous concentration of 4-CIN (Sigma-Aldrich, St. Louis, MO) of 10 mM. Only modest reductions of cellular membrane MCT has been found with lower concentrations of 4-CIN (0.2–0.5 mM) in hippocampal slice preparations and in isolated rat retina. As a consequence, we used a higher in vivo concentration of 4-CIN to ensure inhibition of MCT.

**Exogenous Metabolic Substrates**

Lactate, pyruvate, \( \alpha \)-ketoglutarate, alanine, succinate, or glutamine (Sigma-Aldrich) were diluted in phosphate-buffered saline, and adjusted approximately to pH 7.4. All agents were adjusted to give a final vitreous concentration of 10 mM, being well beyond the saturating level for their respective transporters and the physiological levels found in the retina. Intravitreal injection of the above substrates at 10 mM in control eyes was shown to have no effect on retinal function (data not shown).

**Inhibition of Glutamate–Glutamine Cycle Pathways**

The glutamate–glutamine cycle is intimately linked to metabolic pathways, as summarized in Figure 1. Key reactions facilitating this link involve glutamate production from glutamine (through phosphate-activated glutaminase, [PAG]) and oxidation of glutamate to \( \alpha \)-ketoglutarate through glutamate dehydrogenase (GDH) or the aminotransferases (aspartate aminotransferase, AAT; alanine aminotransferase, ALAT; branched chain aminotransferase, BCAT; γ-aminobutyric acid aminotransferase, GABA-T; and glutamine aminotransferase). The importance of these pathways was studied with 6-diazo-5-oxo-norleucine (DON, 10 mM; Sigma-Aldrich), an inhibitor of PAG, and aminoxyacetic acid (AOAA, 10 mM; Sigma-Aldrich), an inhibitor of the aminotransferases.

**RESULTS**

**Effect of MCT Inhibition**

The rat ERG waveform (Fig. 2A, open circles) is characterized by an initial negativity (a-wave), followed by a corneal positive potential (b-wave) with small waves, termed OPs, found on the rising edge of the b-wave. The application of 4-CIN rapidly attenuated the b-wave, whereas the photoreceptor response was slightly enlarged and delayed (Fig. 2A). OPs were also reduced by 4-CIN, as shown in Figure 2B. The largest functional changes were observed 40 minutes after 4-CIN treatment, for all ERG parameters (Fig. 3). More
Specifically, the P3 amplitude ($R_{mP3}$) was significantly increased by 4-CIN treatment (filled circles, $P < 0.001$) compared with vehicle treatment (Fig. 3A, open circles), whereas phototransduction gain (log $S$) was reduced by 29% ± 5% (Fig. 3B, $P < 0.05$). B-wave amplitude was reduced ($P < 0.001$) by as much as 79.9% ± 4.4% (Fig. 3C), and b-wave implicit times were significantly delayed (Fig. 3D, $P < 0.05$, −32.6 ± 8.0 ms). Figure 3E shows that 4-CIN reduced OP amplitudes at a similar rate and magnitude to the b-wave (80.5% ± 2.4%), whereas OP implicit time was not affected (Fig. 3F). It is likely that the enhanced P3 amplitude reflects unmasking of the photoreceptor response by the diminished and slowed b-wave component.

Effect of Exogenous Metabolic Substrates

To consider the completeness of MCT inhibition, we introduced the exogenous monocarboxylate, lactate at 40 minutes after application of 4-CIN. Exogenous lactate (filled circles) did not significantly affect the P3 amplitude (Fig. 4A, $P = 0.30$ [0.98]), log $S$ (Fig. 4B, $P = 0.31$ [0.87]), or OP amplitude (Fig. 4C, $P = 0.27$ [0.79]) compared with 4-CIN alone (open circles). However, b-wave amplitude was significantly improved by lactate (10 mM, Fig. 4D, filled circles, $P < 0.001$, 24% ± 7%). Lactate loading did not significantly alter b-wave implicit times (Fig. 4E, $P = 0.36$ [0.85]).

If cytosolic lactate was first converted to pyruvate by lactate dehydrogenase (LDH, see schematic in Fig. 4), $^{23}$ then exogenous pyruvate should induce recovery similar to that found with lactate. As expected, pyruvate (10 mM) loading mimicked the effects of lactate, with a significant improvement in b-wave amplitudes of up to 27% ± 8% (Fig. 5A, filled circles, $P < 0.001$) compared with 4-CIN alone (open circles). Analogous to previous findings for lactate loading, pyruvate supply had no effect on P3 amplitude ($P = 0.54$ [0.85]), log $S$ ($P = 0.13$ [0.93]), b-wave implicit time ($P = 0.62$ [0.73]), or OP amplitude ($P = 0.56$ [0.67]), compared with 4-CIN alone (data not shown).

As 4-CIN blocks both cellular and mitochondrial uptake of pyruvate, $^{23}$ substrate supply to the mitochondrial enzymes pyruvate dehydrogenase (PDH) and pyruvate carboxylase (PC) should be impaired. $^{54,55}$ An alternate pathway is for pyruvate to enter mitochondria through transamination to α-ketoglutarate by ALAT (Fig. 5, schematic). Figure 5B shows that exogenous α-ketoglutarate significantly improved the b-wave amplitude (filled squares, $P < 0.001$) compared with 4-CIN alone (open circles). The 27% ± 8% b-wave recovery is comparable to that afforded by lactate (24% ± 7%) and pyruvate (27% ± 8%), suggesting that that functional recovery likely involves the enzymatic pathway catalyzed by ALAT. P3 amplitude ($P = 0.53$ [0.78]), log $S$ ($P = 0.64$ [0.72]), b-wave implicit time ($P = 0.64$ [0.73]), and OP amplitude ($P = 0.56$ [0.78]) were unaltered by the introduction of α-ketoglutarate compared with 4-CIN alone (data not shown).

α-Ketoglutarate can enter the TCA cycle$^{56}$ or act as a precursor for the neurotransmitter glutamate.$^{5}$ To differentiate between the metabolic and neurotransmitter mechanisms, we introduced alanine, which is an essential nitrogen donor for

![Figure 2](iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933227/)
We reasoned that if alanine and α-ketoglutarate were used to replenish neurotransmitter glutamate pools, then alanine supply should induce greater b-wave recovery than any of our other substrates (Fig. 6, schematic). In contrast to this prediction, we found that exogenous alanine only improved the b-wave amplitude (Fig. 6A, filled circles, P < 0.001) by 25% ± 10%, which is similar to other substrates. In addition, the P3 amplitude (P = 0.56 [0.76]), log S (P = 0.06 [0.90]), b-wave implicit time (P = 0.31 [0.86]), and OP amplitude (P = 0.43 [0.67]) were unaltered by alanine application (data not shown).

We also bypassed the aminotransferases by supplying the TCA cycle intermediate succinate, which under normal conditions is derived from the GABA shunt through GABA-T and succinate semialdehyde dehydrogenase (SSAD; Fig. 6, schematic).60 The introduction of succinate significantly improved b-wave amplitudes (Fig. 6B, filled squares, P < 0.001) consistent with a contribution of the GABA shunt to metabolism. However, the level of recovery (26% ± 8%) did not exceed the other exogenous substrates. In addition, other functional parameters were unaltered by succinate supply, including P3 amplitude (P = 0.85 [0.59]), log S (P = 0.06 [0.92]), b-wave implicit time (P = 0.60 [0.74]), and OP amplitude (P = 0.52 [0.75]), compared with 4-CIN alone (data not shown).
Association of Pyruvate- and Alanine-Induced Functional Recovery with ALAT

We reasoned that if the pyruvate-induced b-wave recovery involves its transamination to α-ketoglutarate through ALAT, then this recovery should be suppressed by the aminotransferase inhibitor AOAA (Fig. 7, schematic). Consistent with this proposal, Figure 9A shows that, although initially the b-wave amplitude recovered, DON (filled circles) gradually abolished the b-wave recovery compared with glutamine alone (open circles, \( P < 0.01 \)). The OP recovery observed with glutamine was also suppressed by DON application (\( P < 0.01 \)), whereas little change was observed for P3 amplitude (\( P = 0.34 \) [0.68]), log S (\( P = 0.19 \) [0.79]), or b-wave implicit time (\( P = 0.09 \) [0.81], data not shown).

Once converted from glutamine to glutamate, glutamate can replenish neurotransmitter pools and/or enter metabolic pathways. We predicted that if glutamine-induced recovery occurs through a metabolic mechanism, then inhibiting the aminotransferase reactions (with AOAA) should suppress the glutamine induced b-wave recovery. The inclusion of AOAA with glutamine (Fig. 9A, filled squares) completely suppressed the b-wave (Fig. 9A, open circles, \( P < 0.001 \)) and OP amplitude (\( P < 0.001, \) data not shown) improvement compared with glutamine alone. AOAA also significantly increased P3 amplitude (\( P = 0.01 \)) and further delayed b-wave implicit times (\( P = 8D, P = 0.88 \) [0.57]). Similar to the b-wave, OP amplitude was significantly improved, however the rate of improvement was slower (Fig. 8E, \( P < 0.001 \)).

Under normal conditions, glutamine exported from glia is taken up by neurons to be converted to glutamate by the enzyme PAG.\(^2\)\(^,\)\(^6\)\(^,\)\(^5\) We expected that inhibiting PAG using the reversible inhibitor DON\(^9\)\(^6\)\(^,\)\(^9\) should impair glutamate production and thus blunt the glutamine-induced recovery (Fig. 9, schematic). Consistent with this proposal, Figure 9A shows that, although initially the b-wave amplitude recovered, DON (filled circles) gradually abolished the b-wave recovery compared with glutamine alone (open circles, \( P < 0.01 \)). The OP recovery observed with glutamine was also suppressed by DON application (\( P < 0.01 \)), whereas little change was observed for P3 amplitude (\( P = 0.34 \) [0.68]), log S (\( P = 0.19 \) [0.79]), or b-wave implicit time (\( P = 0.09 \) [0.81], data not shown).

The Glutamate–Glutamine Cycle and Functional Recovery from 4-CIN

To directly consider interactions between the glutamate–glutamine cycle and metabolism, we supplied the glutamate precursor glutamine, which is known to be an excellent metabolic substrate.\(^25\)\(^,\)\(^46\)\(^,\)\(^61\)\(^,\)\(^62\) Exogenous glutamine supply resulted in a large improvement of the b-wave amplitude as shown by the representative waveforms in Figure 8A (filled circles) compared with 4-CIN alone (open circles). After glutamine supply, the P3 amplitude (\( P < 0.001, \) data not shown) was significantly improved, whereas no improvement was observed for log S (Fig. 8B, \( P = 0.06 \) [0.96]). A maximum b-wave amplitude improvement of \( 62\% \pm 3\% \) was observed at 100 minutes after glutamine supply (Fig. 8C, \( P < 0.001 \)). However, b-wave implicit time was not improved compared with 4-CIN alone (Fig. 8D, \( P = 0.88 \) [0.57]). Similar to the b-wave, OP amplitude was significantly improved, however the rate of improvement was slower (Fig. 8E, \( P < 0.001 \)).

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finding for a reduced log \( S \), together with the inability of exogenous substrates to restore this parameter, suggests that 4-CIN induced some metabolic impairment in photoreceptors, as log \( S \) is sensitive to complete metabolic inhibition.\(^1\) Hence, it is unlikely that 4-CIN spares photoreceptors. Alternatively, the b-wave and OP loss could reflect an impairment of neurotransmission between photoreceptors and ON-bipolar cells,\(^2,5\) which can occur through a direct effect of 4-CIN on neurotransmission or, indirectly, as a corollary of glutamate oxidation for metabolism. Indeed, 4-CIN has been shown to reduce glutamate levels in the retina.\(^17,69\) Our experiments using exogenous substrates provide evidence that an impairment of neurotransmission contributes to the postreceptoral dysfunction.

**Exogenous Metabolic Substrates and Reversibility of MCT Blockade**

We found that the metabolic deficit induced by 4-CIN in the rat retina could be partially ameliorated with various exogenous metabolic substrates, including pyruvate, lactate, \( \alpha \)-ketoglutarate, alanine, succinate, and glutamine. Although this group is limited, they represent important metabolic intermediates in the retina (see Fig. 1).\(^20\) The similar magnitude of b-wave amplitude recovery induced by these substrates (with the exception of glutamine) is consistent with a common constraint for the magnitude of functional improvement. Given the similar magnitude of b-wave recovery observed with pyruvate and lactate, it seems unlikely that 4-CIN affected the interconversion between lactate and pyruvate (LDH).\(^53\)

As lactate and pyruvate depend on the very transporters that we were inhibiting, it is not surprising that full b-wave recovery was not observed. At both the cellular and mitochondri...
The Glutamate–Glutamine Cycle and Retinal Function

The large b-wave and OP recovery from 4-CIN afforded by glutamine could be expected, given the effectiveness of this compound as a metabolic substrate in the brain and retina. More important, that glutamine-induced b-wave and OP recovery was blunted by DON confirmed that the glutamate–glutamine cycle provides fuel for retinal metabolism, through conversion to glutamate. PAG has been localized throughout the rat retina in both neurons and Müller cells. The greater functional recovery with glutamine compared with other substrates could also arise from its ability to contribute to neurotransmitter glutamate pools and thereby synaptic neurotransmission. Our finding that the glutamine-induced b-wave/OP recovery was completely suppressed by AOAA provides strong evidence that the glutamate produced from glutamine first entered the TCA cycle, rather than the neurotransmitter pools. Moreover, the entry of glutamate carbon skeletons to the TCA cycle occurs through the aminotransferase reactions.

It is worth considering the possible reasons behind the delayed effect of DON on the b-wave recovery with glutamine compared with AOAA. This outcome may reflect the existence of an alternate pathway for glutamate production from glutamine. Indeed, the inhibition of PAG by DON could have been bypassed by a two-step reaction for glutamate synthesis involving glutamine aminotransferase. However, the presence of glutamine transaminase has yet to be confirmed in the retina. In contrast, AOAA inhibits many enzymes, including AAT, ALAT, BCAT, ornithine aminotransferase, GABA-T, and glutamine transaminase. Such a mechanism may explain why AOAA suppressed glutamine-induced functional recovery from 4-CIN more rapidly than did DON.

That the aminotransferase reactions are involved in the glutamine recovery also provides a parsimonious explanation for the selective b-wave/OP loss found in our study. Specifically, 4-CIN application in the retina leads to a reduction of glutamate and glutamine levels and significantly elevated aspartate levels, whereas ATP levels remained unperturbed. This pattern of change in the amino acids signals glutamate oxidation, with the specific elevation in aspartate implicating a shift in AAT activity. By oxidizing glutamate, α-ketoglutarate can be supplied to the TCA cycle.

Consistent with this idea, when the aminotransferases were inhibited by AOAA, the b-wave recovery afforded by pyruvate and alanine was completely suppressed. In addition, after sufficient time in the presence of AOAA or DON, glutamine was unable to ameliorate the functional deficit caused by 4-CIN. Together, these findings suggest that glutamate oxidation through the aminotransferase reactions can alleviate some of the metabolic deficit induced by 4-CIN. However, glutamate oxidation depletes the neurotransmitter pool; thus, the selective postreceptoral loss reflects the interplay between the metabolic and neurotransmitter glutamate pools. Indeed, inhibiting glutamate recycling through glutamine synthetase leads to functional deficits similar to those observed in our study.

An important implication of the ability of AOAA to suppress pyruvate-, alanine-, and glutamine-induced b-wave recovery is that the aminotransferase reactions are more important for in vivo glutamate oxidation than is GDH. Although, under normal conditions, glutamate oxidation occurs largely through GDH, gluta-
our interpretation, Farinelli and Nicklas63 have shown that inhibition of AAT reduces the $^{14}\text{CO}_2$ production by [1-14C]glutamate by more than 70%. In addition, the relative activity of GDH84 is quantitatively less than ALAT85 which is, in turn, an order of magnitude less than AAT82,86. However, the localization of GDH in photoreceptor inner segments, terminals, and nuclei and, to a lesser extent, in Müller cells, suggests that GDH is crucial in photoreceptor function.68 Nevertheless, our study suggests that the aminotransferases are largely responsible for the postreceptoral recovery from 4-CIN.

In summary, we found that normal MCT is important for in vivo visual function. Our findings provide evidence that the functional loss induced by inhibition of MCT using 4-CIN involves some metabolic deficit in the rat retina. Such a metabolic deficit is manifest more in the postreceptoral b-wave and OP components of the ERG. This reduction in b-wave amplitude may involve a reduction of glutamate neurotransmission between photoreceptors and ON-bipolar cells. This does not arise from a direct effect of 4-CIN on neurotransmission, rather it reflects preferential routing of amino acid carbon skeletons to non-neuronal metabolic pathways, which in turn reduces glutamate availability for neurotransmission. These findings have important implications for our understanding of retinal neurotransmission as it relates to the metabolic status of the retina.

References

Retinal Metabolic Function