Glycolytic and Oxidative Metabolism in Relation to Retinal Function

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ABSTRACT Measurements of lactate production and ATP concentration in superfused rat retinas were compared with extracellular photoreceptor potentials (Fast PIII). The effect of glucose concentration, oxygen tension, metabolic inhibition, and light were studied. Optimal conditions were achieved with 5–20 mM glucose and oxygen. The isolated retina had a high rate of lactate production and maintained the ATP content of a freshly excised retina, and Fast PIII potentials were similar to in vivo recordings. Small (<10%) decreases in aerobic and anaerobic lactate production were observed after illumination of dark-adapted retinas. There were no significant differences in ATP content in dark- and light-adapted retinas. In glucose-free medium, lactate production ceased, and the amplitude of Fast PIII and the level of ATP declined, but the rates of decline were slower in oxygen than in nitrogen. ATP levels were reduced and the amplitude of Fast PIII decreased when respiration was inhibited, and tiese changes were dependent on glucose concentration. Neither glycolysis alone nor Krebs cycle activity alone maintained the superfused rat retina at an optimal level. Retinal lactate production and utilization of ATP were inhibited by ouabain. Mannose but not galactose or fructose produced lactate and maintained ATP content and Fast PIII. Iodoacetate blocked lactate production and Fast PIII and depleted the retina of ATP. Pyruvate, lactate, and glutamine maintained ATP content and Fast PIII reasonably well (>50%) in the absence of glucose, even in the presence of iodoacetate. Addition of glucose, mannose, or 2-deoxyglucose to medium containing pyruvate and iodoacetate abolished Fast PIII and depleted the retina of its ATP. It is suggested that the deleterious effects of these three sugars depend upon their cellular uptake and phosphorylation during the blockade of glycolysis by iodoacetate.

INTRODUCTION

Metabolism of the mammalian retina has been studied extensively (Futterman and Kinoshita, 1959; Graymore and Tansley, 1959; Cohen and Noell, 1960 and 1965; see reviews by Graymore [1970], Sickel [1972], and Winkler [1981]). The retina possesses many interesting metabolic properties, including high rates of oxygen consumption and lactic acid production. Electrical activity of the isolated mammalian retina depends on available glucose (Ames and Gurian, 1963; Winkler, 1972). Intravenous injection of iodoacetate (IAA),

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a potent inhibitor of sulfhydryl enzymes such as triose phosphate dehydrogenase, leads to loss of electrical activity and the selective disappearance of the rod photoreceptor cells (Noell, 1959). Energy for metabolic and electrical activity comes largely from the oxidative breakdown of glucose (Noell, 1959; Futterman and Kinoshita, 1959; Cohen and Noell, 1960; Ames and Gurian, 1963; Winkler, 1974 and 1975). Glycolysis alone is capable of supporting electrical activity in photoreceptor cells of the isolated rat retina; however, this activity is dependent upon the experimental conditions and the species of animal studied (Winkler, 1975).

The importance of glucose in the retina has raised the question: is there an important role for glycolytically derived ATP in the function and survival of a photoreceptor cell? This question remains unanswered, because it has been impossible to differentiate the ATP synthesis from glycolysis or respiration. One approach to this problem is to determine whether the mammalian retina can utilize substrates other than glucose for the production of ATP by aerobic mechanisms that do not involve glycolysis (Futterman and Kinoshita, 1959; Hopkinson and Kerly, 1959; Cohen and Noell, 1960; Catanzaro et al., 1962). The experiments described in this report were undertaken to analyze effects of sugar substitutes, Krebs cycle intermediates, glucose and oxygen deprivation, and metabolic poisons on the amplitude and wave form of the extracelluarly recorded receptor potential. An attempt was made to correlate these observations with parallel measurements of retinal lactic acid production and ATP content in darkness and light. The primary objective was to study glycolysis and respiration as related to photoreceptor cell function when only one of these pathways at a time is operative.

MATERIALS AND METHODS

A. *Surgery*

Adult albino rats weighing between 175 and 300 g were maintained on a 12 h of light–12 h of darkness cycle and fed standard lab chow. The retina was isolated from the other ocular tissues by methods that have been described (Winkler, 1972). In brief, the rat eyeball was proptosed by placing forceps around the optic nerve close to its exit from the eye. The globe was transected along the equator and the cornea and lens were removed. The attachment of the optic nerve to the retina was severed by pressing upward with the forceps, and the retina was progressively detached from the pigment epithelium. Finally, the retina was completely detached from the pigment epithelium by the continued upward movement of the forceps. The entire isolated tissue (6–8 mm) was then deposited in a Petri dish filled with perfusion fluid ready to be mounted in the incubation chamber. The surgical procedure was routinely completed in less than 1 min. The rats were dark adapted for at least 2–4 h and surgical procedures were carried out in dim red light.

B. *Electrical Measurements*

The isolated retina was mounted in a specially designed perfusion chamber (Winkler, 1972 and 1978), which permitted electrophysiological recordings in conjunction with measurements of lactic acid production in a light-tight, copper-shielded cage. Light-induced transretinal potentials were recorded differentially between two nonpolariz-

able Ag-AgCl electrodes. Intraretinal recordings of the receptor potential (Fast PIII) were also made with a saline-filled micropipette that was inserted into the retina and positioned about 100 μm from the distal tips of the outer segments. All signals were amplified and displayed, either on an oscilloscope (these signals were photographed) or on a penwriter. Diffuse, white-light stimuli of fixed, brief duration and variable intensity were provided by a Grass Instrument Co. (Quincy, Mass.) model PS-22 photostimulator. The number of photons incident on the retina from the flash of highest intensity was calculated to be $\sim 2 \times 10^{11}$ photons/mm^2. To increase the range of light intensities, neutral-density filters were interposed between the light source and the retina.

C. *Solutions*

The medium for the control or standard solution throughout the experiments was (concentrations in mmol/liter) NaCl, 120; KCl, 5; $NaHCO_3$, 25; D-glucose, 5; $CaCl_2$, 2; $MgSO_4$, 0.5; sodium aspartate, 10. Sodium aspartate was used to block synaptic transmission from the photoreceptor cells to postsynaptic neurons, thereby causing the loss of the *b*-wave of the electroretinogram (ERG) and leaving a well-preserved PIII component, comprised of its Fast and Slow fractions. The solutions were bubbled continuously with a mixture of 95% O_2 and 5% CO_2. pH was 7.4–7.5, and the total osmolarity was ~315 mosmol. Temperature was maintained at 37°C. Alterations in the media were made by equivalent substitution of appropriate constituents. Details of such replacements are given in the text and legends. All chemicals were reagent grade. Control ionic constituents were kept as 10-fold concentrated stock solutions; all other chemicals were weighed out on a daily basis. D(+)-galactose, β-D(−)-fructose, and 2-deoxyglucose and sucrose were purchased from Sigma Chemical Co., St. Louis, Mo., as were lithium lactate, pyruvic acid, and the various other Krebs cycle intermediates. Glutamine and glutamic acids were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio.

D. *Biochemical Measurements*

The major metabolic components measured were retinal lactic acid production and ATP content. Lactic acid production was measured under two different incubation conditions. Firstly, lactate was measured simultaneously with light-induced electrical activity from a single isolated retina superfused with a continuously recirculating volume of 10 ml or less in order to sufficiently concentrate the lactate for determination by the method of Barker and Summerson (1941). Samples of the perfusion fluid (0.25 ml) were removed for analysis every 5 min with a constriction pipette. Solutions were changed with no discontinuity of flow past the retina. The new solution was allowed to circulate for ~0.5–1 min, ensuring removal of any traces of lactate from the former solution, before its volume was standardized and a "time-zero" sample removed. That the washout was complete was indicated by the fact that water and the time-zero sample had similar absorbancies. Thereafter, samples were again taken at 5-min intervals, and a duplicate sample was always removed at the end of perfusion with a particular test solution. Secondly, lactate production was measured from three retinas incubated together in an apparatus similar to that used by Merriam and Kinsey (1950) for lens culture but which was modified for retinal incubations. In this experimental arrangement, the three retinas were placed in a 50-ml flask containing 10 ml of medium, and samples were removed for determination of lactate as described above. This latter system enabled the simultaneous measurement of lactic acid from as many as six groups of retinas, thereby facilitating data acquisition. Moreover, there were no significant differences in lactate production from retinas maintained under

the two different incubation conditions. After incubation, retinal dry weights were obtained after overnight baking at 77°C to constant weight; the average dry weight of the retina was 1.5 mg. Statistical analyses of the slopes of lactate curves generated by regression analysis indicated that differences in lactate production as low as 5–10% could be resolved. ATP content was measured in pairs of retinas incubated in the culture system and then homogenized in 0.20 ml of 12% trichloroacetic acid. ATP was determined by a modification of a Sigma Chemical Co. (Kit 366-UV) enzymatic assay.

RESULTS

I. *Lactic Acid Production and ATP Content of Superfused Albino Rat Retinas*

A. GENERAL CONSIDERATIONS, EFFECTS OF LIGHT AND INHIBITORS OF SYNAPTIC TRANSMISSION Lactic acid production from a single, superfused rat retina was measured while light-induced electrical activity was recorded. Initial experiments established the conditions necessary for linear accumulation of lactate during steady superfusion with the control solution. Fig. 1 (*left*) shows that a linear rate of aerobic (and, although not shown here, anaerobic) lactate production was obtained from a dark-adapted retina, provided the medium was replaced every 30 min. When the medium was not replaced, the linearity of lactate production was not maintained beyond 75 min, as can be seen in the right graph of Fig. 1. This plateau in lactate production was not due to deterioration of the retina, since a linear accumulation of lactate again was observed after a change of solution at 105 min. Also, when samples were removed every 15 min, linearity of lactate production typically was observed over this entire period of time. It is possible that the decline in the rate of lactate production in the absence of any solution change results from the reduction in the total volume, as was observed by Cohen and Noell (1960). When 0.25-ml samples were taken every 5 min, there was a 40% reduction in total volume at the end of a 75-min incubation. Therefore, in the subsequent experiments, all solutions were routinely drained and replaced every 30 min. An identical method of solution change was employed in the culture system to mimic the conditions established for the superfused retina. When a dark-adapted retina was incubated for 60 min in the culture apparatus and then transferred, in darkness, to the perfusion system, electrical responses to light were observed that were comparable to those recorded from retinas superfused immediately after isolation, demonstrating the usefulness of this incubation system for preservation of retinal function.

Fig. 2 illustrates the effects of light on aerobic lactate production from superfused retinas. In this experiment, the retina was superfused with control medium for three consecutive 30-min periods in a dark-light-dark sequence. Electrical responses were stable throughout the initial 30 min of perfusion. The solution was then drained and replaced, a new reference sample was taken, and the retina was exposed to room light for 30 min. During this period of strong light adaptation, Fast PIII was not observed. Thirty minutes later the room light was turned off, the solution was replaced, and further samples were taken. No recovery of Fast PIII was observed, probably because the

visual pigment molecules bleached by the light do not regenerate when the isolated rat retina is dark adapted (Delmelle et al., 1975).

The averaged data in Fig. 2 show that aerobic lactate is not appreciably decreased by light, the rates in darkness and in light being 1,294 ± 56 and 1,215 ± 50 nmol/mg dry wt·h^{-1} (n = 8, mean ± SE), respectively. On the basis of unpaired statistical analyses, considering the average of each rate independently, the difference in lactate production in darkness and in light is not significant at the P = 0.05 level. However, the 6% decrease in lactate

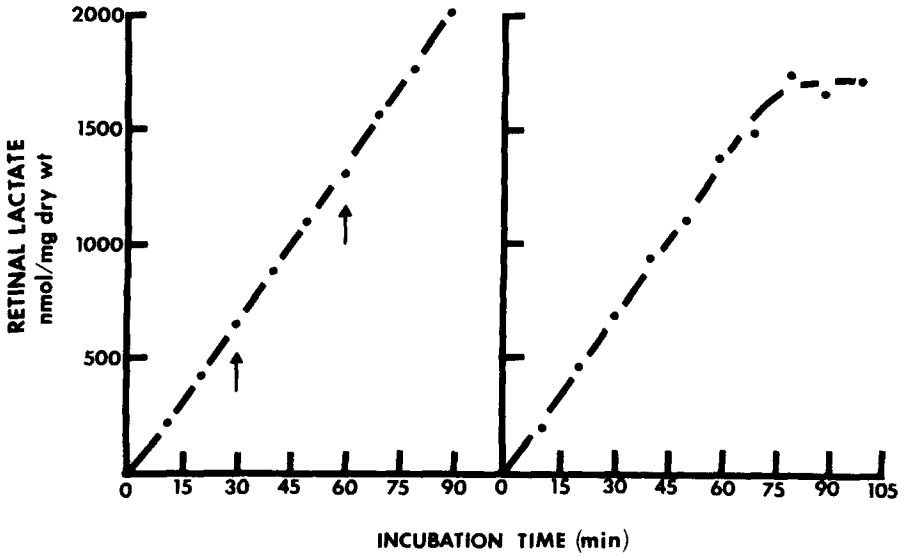

FIGURE 1. Aerobic lactate production from dark-adapted retinas that were continuously superfused with (*left*) and without (*right*) a change of solution every 30 min. The arrows indicate the times at which the solutions were changed according to the methods described in the text. Although samples of the superfusion medium for lactate determination were collected every 5 min, for presentation purposes only the values at 10-min intervals are shown. The amount of lactate produced during any given 10-min period was added to the previous value to yield a new total. Each point represents the average of five experiments.

production is significant at the P = 0.025 level when the value of t is calculated according to paired statistical analysis (t = average difference/SE). Thus, the average difference (±SE) in lactate production in darkness and in light is 78 ± 30 nmol lactate/mg dry wt·h^{-1}, and t = 2.60. Light also had a small effect on anaerobic lactate production when measured under the same conditions with 2 mM KCN added to the control medium. In these experiments (n = 4), average (±SE) anaerobic lactate production in the dark and light was 2,009 ± 90 and 1,836 ± 94 nmol/mg dry wt·h^{-1}, respectively. This difference was significant at the P = 0.025 level, using paired statistical analysis. It was

possible that there were early, larger effects of light on lactate accumulation, i.e., within the first minute, which may not have been detected because the first sample was removed after 5 min. Accordingly, in several experiments the room lights were turned on without a change in solution and samples were taken every 15 s during the first minute. Lactate production during this initial minute of light adaptation was not significantly different from the rate found 5 min later.

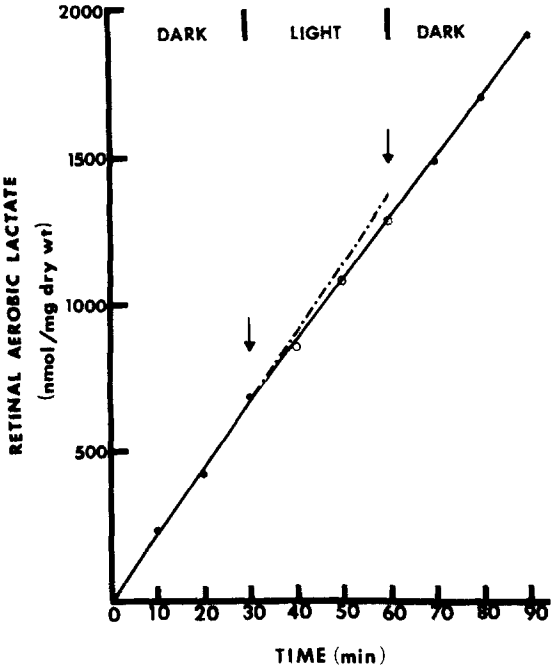

FIGURE 2. The effect of a 30-min period of strong light adaptation (open circles) on aerobic lactate production from superfused rat retinas. The arrow at 30 min indicates when the room lights were turned on, and the arrow at 60 min when they were turned off. Data are plotted as in Fig. 1, with successive lactate values being added to the previous total. Each point is the average of eight experiments. Standard deviations (not shown) were <12% of the measured values. The solid line is the best visual fit to the data points. The dashed line between 30 and 60 min is an extension of the rate of lactate production in the dark during the initial 30-min period of incubation and provides a comparison with the rate obtained in the light and in the dark after the lights were turned off.

Table I shows that there were no significant differences in ATP content between freshly excised dark- and light-adapted retinas nor between these and retinas that had been incubated in control medium for 30 min. This same level was maintained after 120 min in control medium. These results illustrate that retinal ATP content is stable under these control conditions.

Because sodium aspartate was included in the control medium to isolate PIII (Fig. 3), it was necessary to determine whether its presence affected the metabolic activities of the retina. It was found that aerobic and anaerobic lactic acid production and ATP content (Table I) in dark- and light-adapted retinas were not significantly affected by aspartate. Moreover, the presence of sodium aspartate did not influence the metabolic and electrical effects of the various compounds tested in the experiments.

TABLE I

RETINAL ATP CONTENT*

Condition	Dark-adapted	Light-adapted
	μmol/gm dry wt	
Freshly excised	9.9±0.3 (7)	10.0±0.3 (8)
Control medium	9.7±0.4 (7)	9.3±0.5 (6)
Control medium without aspartate	10.2±0.5 (8)	10.3±0.3 (8)

* Incubations were for 30 min. Results are expressed as mean ± SE. The number of experiments is indicated in parentheses.

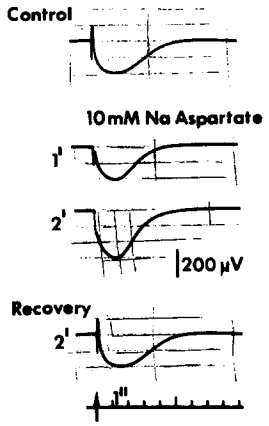

FIGURE 3. The effect of 10 mM sodium aspartate on ERG potentials. The top trace is the control ERG before the addition of aspartate. The middle two tracings show the early effects of this agent. The bottom trace shows the reversibility of these effects. The principal effect of sodium aspartate appears to be the selective loss of the *b*-wave of the ERG. The light flash is indicated by the arrow; time markings are in seconds.

B. DEPENDENCE ON SUBSTRATE CONCENTRATION AND EFFECTS OF METABOLIC INHIBITORS As shown in Table II, lactate production was high in both the aerobic and anaerobic (2 mM KCN) states when the glucose concentration was 20 or 5 mM. Lactic acid production was cut to less than half when the glucose concentration was only 1 mM. The Pasteur effect decreased with decreasing glucose concentration. Mannose (5 mM) replaced glucose in both

aerobic and anaerobic lactic acid production at a level equivalent to slightly less than 5 mM glucose. Galactose and fructose were not substrates of glycolysis. IAA virtually abolished lactic acid production.

Figs. 4 and 5 show the effects of glucose-free media on aerobic and anaerobic lactate production, respectively, after preincubation in media containing 20, 5, and 1 mM glucose. Aerobic lactic acid production ceased immediately when a glucose-free medium was introduced. When glucose was added back, lactic acid production resumed at rates only slightly lower than the control values. Most of the lactic acid produced anaerobically in glucose-free medium occurred during the first 10 min of incubation (Fig. 5), and the amount produced depended upon the concentration of glucose during the control period.

The ATP levels of the whole retina (Table III) under aerobic conditions were highest for 20 and 5 mM glucose. The ATP concentration was lower anaerobically than aerobically for all three glucose concentrations, and the

TABLE II

RETINAL LACTATE PRODUCTION*

Condition	Aerobic	Anaerobic
	nmol/mg dry wt·h^{-1}	
20 mM Glucose	1,591±30 (35)	2,658±68 (13)
5 mM Glucose	1,545±43 (18)	2,234±72 (13)
2 mM Glucose	1,064±62 (4)	1,493±86 (4)
1 mM Glucose	678±65 (7)	917±58 (3)
5 mM Mannose	1,177±65 (4)	1,490±72 (5)
5 mM Fructose	65±18 (6)	145±27 (6)
5 mM Galactose	38±12 (6)	44±13 (6)
5 mM Glucose with 2 mM IAA	50±15 (6)	55±12 (6)

* Incubations were for 60 min. Results are expressed as mean ± SE. The number of experiments is indicated in parentheses.
IAA, iodoacetate.

extent of this decrease was dependent on the concentration of glucose in the medium. Mannose (5 mM) maintained the aerobic ATP level at a slightly lower value than did 5 mM glucose; fructose and galactose were less effective. ATP concentrations were also extremely low in the presence of IAA and when 2-deoxyglucose was added to glucose-free medium.

The rate of loss of retinal ATP was studied as a function of different metabolic interferences, and Fig. 6 shows that when IAA, 2-deoxyglucose, or cyanide is added to glucose-free medium the decline in retinal ATP content is faster than the decline in glucose-free medium alone. Indeed, in the presence of each of these metabolic poisons the retina loses within 5 min >50% of its ATP content. The extent of recovery of ATP levels was also investigated by transferring retinas to glucose-containing medium after the 30-min period of metabolic inhibition. ATP levels recover to 80% of their control levels within several minutes after glucose is resupplied to glucose-depleted retinas, and they recover to ~60% of the preincubation value after incubation with cyanide

and 2-deoxyglucose. No recovery of retinal ATP content is observed after incubation with IAA when glucose is the substrate.

C. EFFECTS OF INHIBITION OF THE SODIUM-POTASSIUM ATPASE The effect of ouabain on lactic acid production and ATP content was investigated under

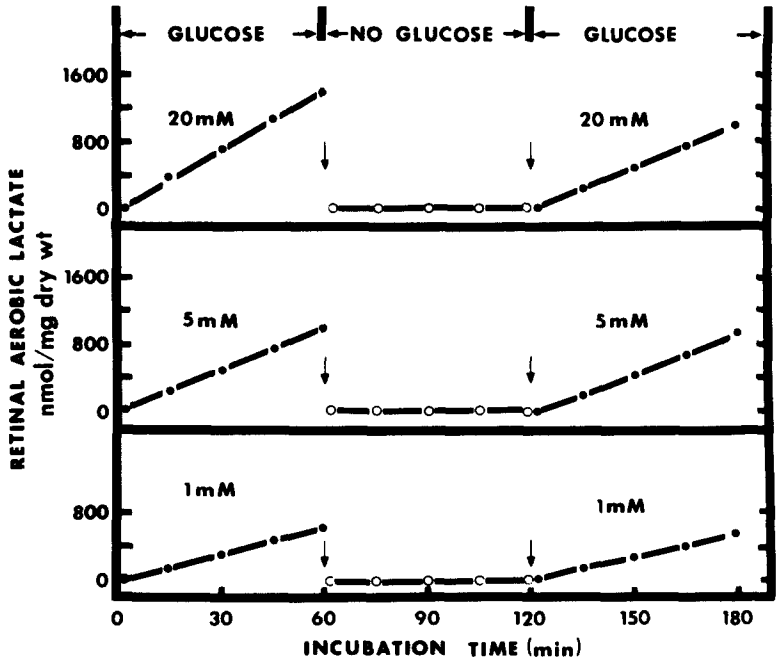

FIGURE 4. Effect of glucose-free medium on aerobic lactate production as a function of different preincubation concentrations of glucose. Retinas were incubated in the presence of 20, 5, or 1 mM glucose for 60 min. Then (at the arrows) a glucose-free solution was introduced for 60 min. Finally (at the arrows), media with the indicated glucose concentrations were reintroduced. During the entire period of incubation, samples for lactate determination were collected at 5-min intervals, although only values obtained at 15-min intervals are shown. The amount of lactate produced during any 15-min period was added to the previous value to yield a new total during superfusion with a particular solution. However, in contrast to Fig. 1, when the solutions were changed the new lactate totals were not added to the previous amount but instead started from their respective "time-zero" values as defined in the text. This accounts for the "breaks" in the graph of lactate production when the control media were replaced with the glucose-free media. Each point is the average of seven experiments.

the premise that a very high proportion of ATP production is used for the active movement of ions across the cell membrane. The results in Table IV show that ouabain decreased aerobic and anaerobic lactate accumulation by 61 and 51%, respectively. However, these substantial effects were not accom-

panied by comparable reductions in retinal ATP content in dark- or light-adapted retinas (Table V).

II. *Dependence of the Receptor Potential (Fast PIII) on the Supply of Glucose and Oxygen in Superfused Albino Rat Retinas*

Although lactic acid production and ATP content of the retina depended greatly on the glucose concentration (Tables II and III), Fast PIII was in all respects the same under different glucose concentrations between 1 and 20 mM. Experiments were performed with 1 mM glucose for 1 h without a significant change in amplitude, wave form, or latency of Fast PIII. Of the

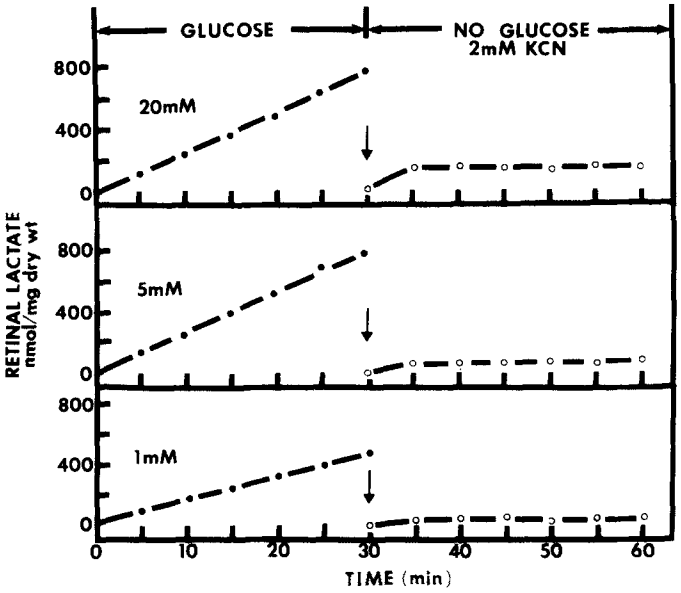

FIGURE 5. The effect of simultaneously removing glucose and adding 2 mM KCN on lactate production from superfused retinas. Retinas were first incubated in media containing 20, 5, or 1 mM glucose for 30 min. At the arrows the solutions were changed and anaerobic conditions were established. Lactate production is plotted as in Fig. 4. Each point is the average of at least three experiments.

various carbohydrates tested as glucose substitutes, only mannose was able to support the amplitude of Fast PIII.

Dependence of Fast PIII upon glucose concentration in the medium became evident when oxygen was replaced with nitrogen (Fig. 7). During 30 min of anoxia, the amplitude of Fast PIII decreased by only 20% when the glucose concentration was 20 mM; it decreased by 60 and 80%, respectively, when the glucose concentration was 5 and 1 mM. The support of Fast PIII by anaerobic glycolysis with 20 mM glucose is paralleled by good survival of Slow PIII, but is in contrast to the support of the *b*-wave and ganglion cell activity (Winkler,

[1972] and unpublished results). In the absence of oxygen these latter activities do not survive in the superfused retina. Also, the absence of bicarbonate, which depresses lactic acid production in the rat retina (Winkler et al., 1977), made the superfused retina much more susceptible to anoxia in the presence of 20 mM glucose than under the control, bicarbonate-containing condition.

TABLE III

RETINAL ATP CONTENT*

Condition	Aerobic	Anaerobic
	μmol/g dry wt	
20 mM Glucose	9.8±0.5 (7)	6.0±0.5 (8)
5 mM Glucose	10.2±0.4 (9)	4.3±0.3 (9)
1 mM Glucose	7.9±0.4 (6)	2.6±0.3 (9)
No substrate	3.7±0.1 (4)	2.3±0.2 (4)
No substrate with 10 mM 2-DOG	3.2±0.2 (6)	1.9±0.2 (3)
No Substrate with 2 mM IAA	2.6±0.1 (6)	2.4±0.2 (6)
5 mM Glucose with 10 mM 2-DOG	5.5±0.1 (6)	2.3±0.2 (5)
5 mM Glucose with 2 mM IAA	2.6±0.5 (11)	2.0±0.1 (7)
5 mM Mannose	9.6±0.5 (5)	3.9±0.2 (4)
5 mM Fructose	5.7±0.2 (11)	2.3±0.2 (5)
5 mM Galactose	4.2±0.2 (4)	2.2±0.1 (4)

* Incubations were for 30 min. Results are expressed as mean ± SE. The number of experiments is indicated in parentheses.
2-DOG, 2-deoxyglucose.

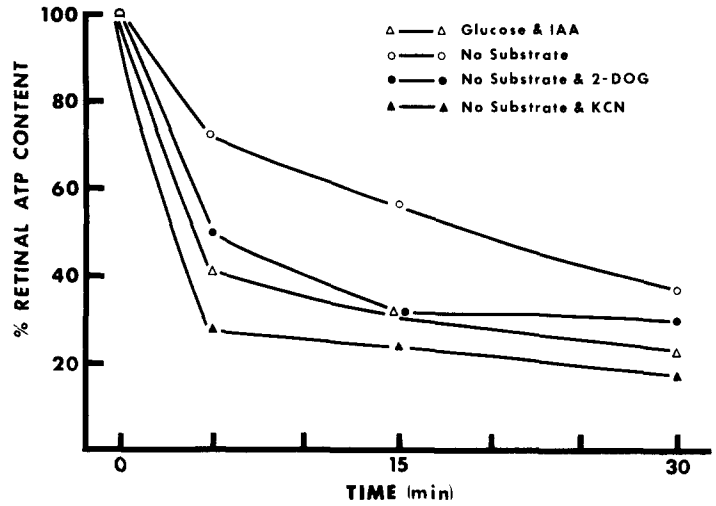

FIGURE 6. A graph of the time-course of change in the relative ATP content of the rat retina as a function of various metabolic interferences. The 100% point at time zero in this and all other graphs of retinal ATP concentration is the value for freshly excised tissue. Each point is the average of at least three experiments. 2-DOG, 2-deoxyglucose.

Fast PIII disappeared within 10 min when bicarbonate was replaced with Tris-phosphate and oxygen was replaced with nitrogen.

Fig. 8 illustrates the effects of glucose-free media on the relative response amplitude of Fast PIII after 1-h incubations in solutions containing different concentrations of glucose. The amplitude of Fast PIII declines in glucose-free medium, the rate of decline being faster the lower the glucose concentration during the control period. After preincubation with 20 mM glucose, the amplitude of Fast PIII decreased by 50% after 20 min in a glucose-free medium, and >1 h was required for its disappearance. In contrast, with a preincubation level of 5 mM glucose, Fast PIII decreased by 50% within 6 min and was not observable after 30 min. As shown in Fig. 9, the rate of loss of Fast PIII was much faster when both glucose and oxygen were withdrawn. The decline in its amplitude now depended only slightly on the preincubation

TABLE IV

RETINAL LACTATE PRODUCTION*

Condition	Aerobic	Anaerobic
	nmol/mg dry wt·h^{-1}	
Control	1,545±83 (18)	2,234±102 (13)
0.1 mM Ouabain	610±35 (6)	1,106±62 (8)

* Incubations were for 60 min. Results are expressed as mean ± SE. The number of experiments is indicated in parentheses.

TABLE V

AEROBIC RETINAL ATP CONTENT*

Condition	Dark-adapted	Light-adapted
	μmol/g dry wt	
Control	10.1±0.6 (3)	9.8±0.9 (3)
0.1 mM Ouabain	9.1±0.5 (3)	9.6±0.2 (3)

* Incubations were for 30 min. Results are expressed as mean ± SE. The number of experiments is indicated in parentheses.

concentration of glucose, with virtual loss of this potential occurring within 10–15 min under all the experimental conditions. Iodoacetate irreversibly abolished Fast PIII in the presence of glucose within 10 min, whereas both 2-deoxyglucose and ouabain caused an even faster loss of Fast PIII (Fig. 10).

III. *Dependence of Fast PIII and Retinal ATP Content on Oxidative Metabolism in Superfused Albino Rat Retinas*

With glucose as the substrate it is not always possible to cleanly separate the contributions of glycolytic and oxidative pathways to the observed electrical phenomena. The following experiments were designed to circumvent this problem by using pyruvate as the substrate for the production of ATP. This means of bypassing glycolysis was used to further investigate the effects of

IAA, since energy production by an intermediate such as pyruvate should be unaffected by the addition of IAA.

In a medium containing 10 mM pyruvate instead of glucose, the amplitude of Fast PIII (and Slow PIII) was maintained at a level >50% relative to the control (5 mM glucose) for at least 60 min (Fig. 11). The addition of 10 mM pyruvate to control medium containing glucose had no effect on Fast PIII. Absence of oxygen in the presence of pyruvate led to the loss of Fast PIII

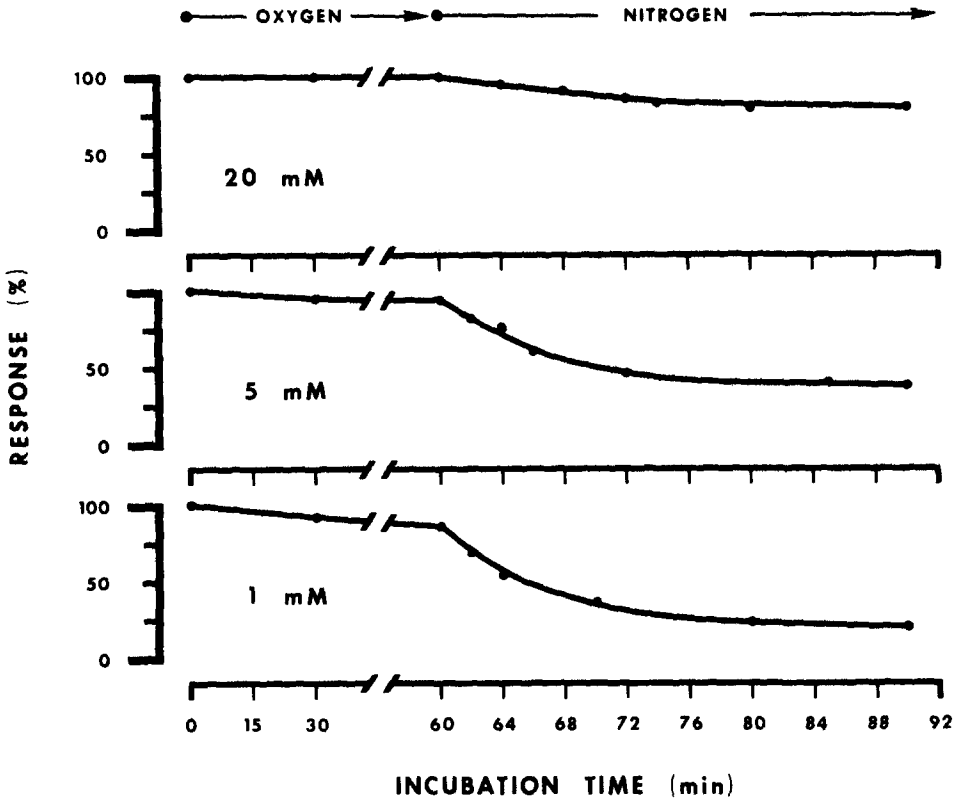

FIGURE 7. Effects of nitrogen on the relative response amplitude of Fast PIII after the incubation of different retinas for 60 min with 20, 5, or 1 mM glucose. The 100% points in this figure and in Figs. 8 and 9 represent the amplitudes of Fast PIII during the initial superfusion in the respective glucose media. Each point is the average of at least five experiments.

within 10 min. Malate, citrate, succinate, fumarate, and glutamate did not replace glucose in the maintenance of either Fast PIII or ATP content, whereas glutamine was at least partially effective. Lactate, however, was as effective as pyruvate. Pyruvate was also able to maintain the *b*-wave for at least 30 min in the absence of glucose.

IAA did not alter the slow decline of Fast PIII in the presence of pyruvate

(Fig. 12), whereas in the presence of glucose it abolished Fast PIII within 5–10 min (Fig. 10). This supports the hypothesis that the principal effect of IAA is related to blocking glycolysis.

It was surprising to find that the addition of glucose (5 mM) to a medium containing pyruvate and IAA abolished Fast PIII within 5 min (Figs. 13 and 14). Recovery was low when glucose was withdrawn or when the retina was washed to remove IAA. Glucose, therefore, appears to have a deleterious effect when its cellular uptake occurs during the blockade of glycolysis by IAA. This

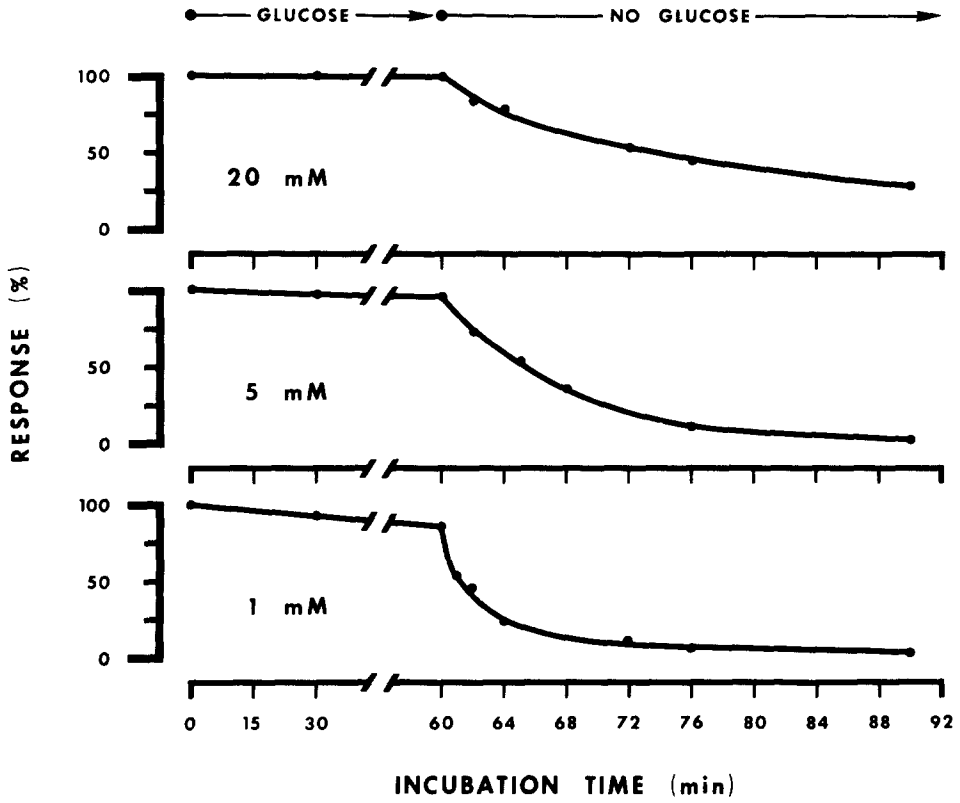

FIGURE 8. Effects of the absence of glucose on the relative response amplitude of Fast PIII after the incubation of different retinas for 60 min with 20, 5, or 1 mM glucose. Each point is the average of five experiments.

effect is not unique to this combination of substrates. It also is observed if either mannose or 2-deoxyglucose is paired with pyruvate. Galactose, fructose, and 3-*O*-methylglucose, however, do not inhibit Fast PIII when paired with pyruvate in the presence of IAA. Glucose, mannose, and 2-deoxyglucose all are sugars that are phosphorylated by hexokinase in the rat retina, a process requiring ATP. It was therefore suspected that ATP produced by the oxidation of pyruvate in the presence of IAA is all utilized in phosphorylating these

three sugars and that the inhibitory effect is due to depletion of high-energy phosphates. This was confirmed by the data of Table VI. ATP content in the presence of IAA was ~80% of that when pyruvate alone was in the medium, but, when either glucose replaced pyruvate or was added to pyruvate-containing medium, the ATP concentration decreased markedly. Essentially the same result was obtained with 2-deoxyglucose. Moreover, as shown in Fig. 15, this depletion of ATP occurred within 5 min, and the rate of decline of ATP was

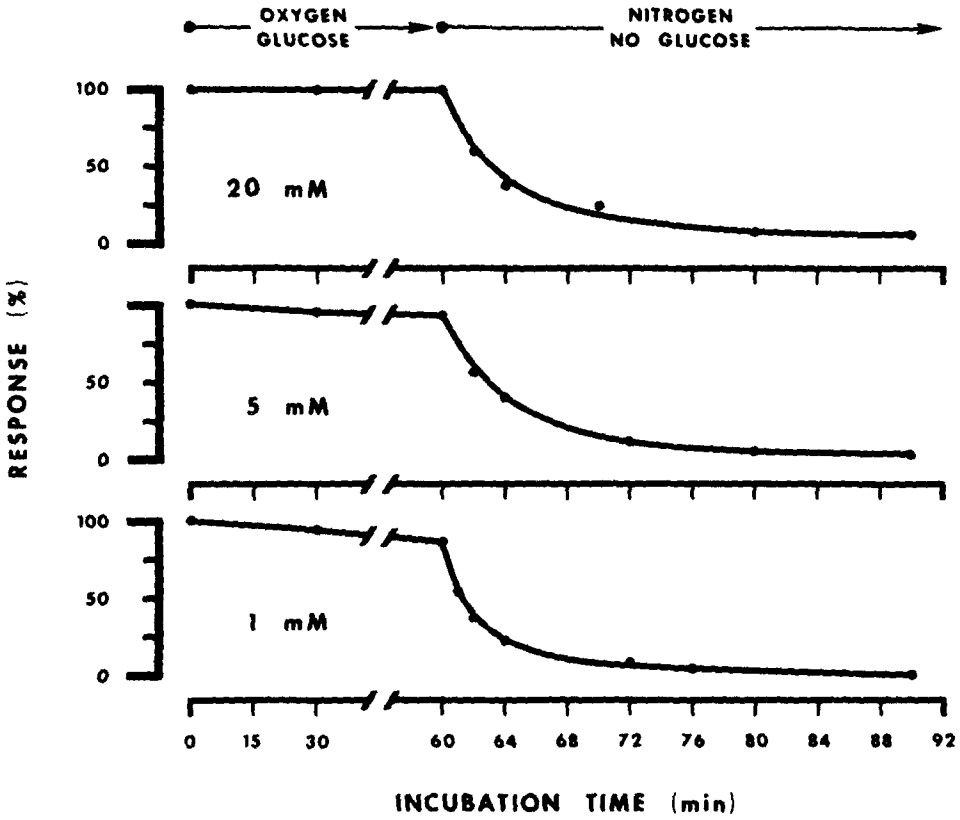

FIGURE 9. Effects of the absence of glucose and oxygen on the relative response amplitude of Fast PIII after the incubation of different retinas for 60 min with 20, 5, or 1 mM glucose. Each point is the average of six experiments.

similar to that observed when IAA was added in the presence of glucose alone (see Fig. 6).

DISCUSSION

In this paper the relationship between metabolism and function has been characterized more completely. Function in the isolated rat retina is defined as the ability of the retina to generate an electrical signal in response to

stimulation by light. The precise electrophysiological criteria chosen to analyze this function were the magnitude and wave form of the intraretinally recorded aspartate-isolated photoreceptor potential, known as Fast PIII in the ERG literature (Faber, 1969). This potential is similar to that measured by Hagins et al., (1970), Arden (1976), and Winkler (1978) in studies of the photovoltages of rat rods. Transretinal recordings were also carried out in the presence and, on occasion, in the absence of aspartate in order to monitor those ERG components (Slow PIII and *b*-wave) of postphotoreceptor cell origin. Measurements of lactic acid production were used to determine the glycolytic capacity of the retina. Although oxygen consumption was not measured in the present series of experiments, it was still possible to obtain qualitative

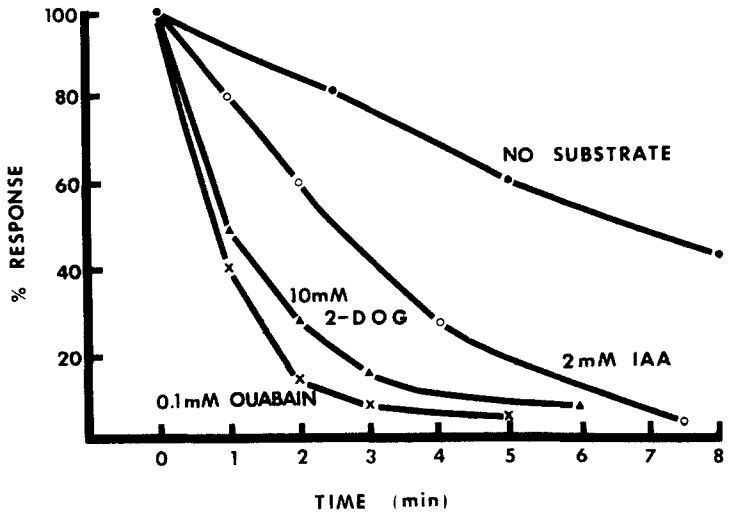

FIGURE 10. The effects of alterations in metabolism, including the absence of substrate and the presence of IAA, 2-deoxyglucose (2-DOG), and ouabain, on the relative response amplitude of Fast PIII from different superfused retinas. The 100% point is the amplitude of Fast PIII during superfusion with the control medium just before the introduction of the test medium. The range of control amplitudes was 150–300 μV. Each point is the average of at least seven experiments.

information about the oxidative capacity of this tissue by comparing lactate production with ATP content under aerobic and anaerobic conditions and by using Krebs cycle intermediates as substrates for energy production. It thus was possible to evaluate the relative contributions of glycolytic and oxidative pathways to retinal function.

Whereas recordings of Fast PIII reflect specifically the electrical response to light of the photoreceptor cells, the metabolic measurements in this study represent the summed activities of all the individual and different cell types, i.e., photoreceptor, bioplar, ganglion, etc., in the retina. There are limitations in extrapolating from the whole retina to the photoreceptor cell layer alone.

However, Graymore, Tansley, and Kerly (Graymore and Tansley, 1959; Graymore, 1960; Graymore et al., 1959) have compared the metabolism of intact rat retinas with the metabolism of dystrophic rat retinas lacking photoreceptor cells and have shown that the specific activities of glycolysis and oxygen uptake in the surviving tissue are reduced to below 50% of those in the intact tissue. This indicates tiat the metabolic rate in the photoreceptor cells is much higher than in the rest of the retina. Although this conclusion derived from the degenerative retina may not be directly applicable to the present study, it does suggest that alterations observed in the glycolytic activity of the whole retina most likely reflect changes in the more active photoreceptor cells.

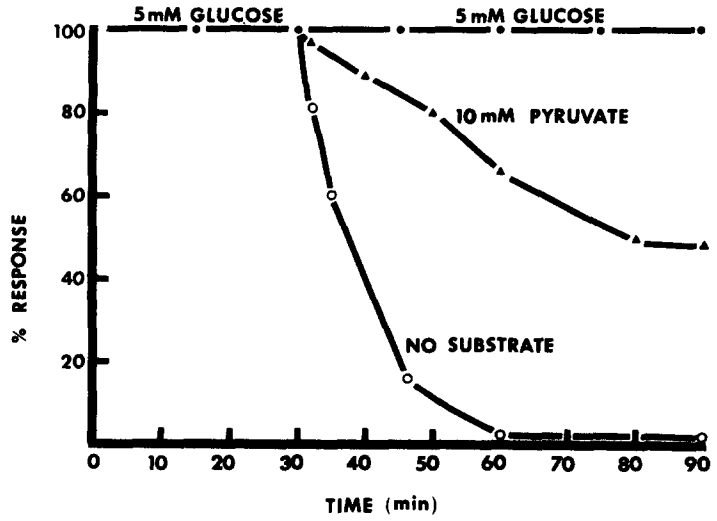

FIGURE 11. Effect of glucose, pyruvate, and no substrate on the relative response amplitude of Fast PIII from different superfused rat retinas. Each point is the average of more than 10 experiments.

The study of the dependence of the electrical activity of the photoreceptor cell on energy metabolism required measurement of metabolic activities under the same conditions that existed during the recording of electrical activity. Lactic acid production and Fast PIII measured from the same superfused retina showed that these activities were stable in the control conditions. This stability was considered an important advantage, because it permitted experiments to be conducted in vitro over several hours without any noticeable loss of function. Also, the incubation media contained a bicarbonate–carbon dioxide buffer system, together with typical extracellular concentrations of all other ionic species. Bicarbonate ions are essential for maintaining high rates of lactic acid production and oxygen consumption in the retina (Hopkinson and Kerly, 1959; Graymore, 1960; Riley, 1965; Riley and Voaden, 1970; Winkler et al., 1977) and for generating the *b*-wave of the ERG (Winkler et

al., 1977). In this way, the variables under study were restricted to type of substrate, oxygen tension, effects of metabolic inhibitors, and lighting conditions. Media containing 5–20 mM glucose in the presence of oxygen provided optimal conditions for incubating the retina. In this medium the rat retina had a high aerobic lactic acid production, maintained an ATP content equal to that of the freshly excised tissue, and generated ERG potentials similar to those recorded in vivo. With concentrations of glucose of 5 mM or greater, aerobic lactate production was maximal and was comparable to other published rates of lactic acid production in whole mammalian retinas (Graymore, 1959; Hopkinson and Kerly, 1959; Glocklin and Potts, 1965; Riley and

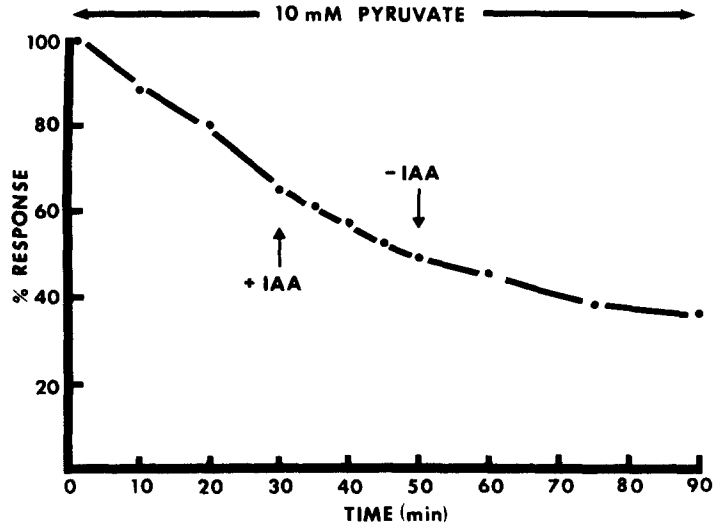

FIGURE 12. Effect of adding IAA (2 mM) in the present of pyruvate on the relative response amplitude of Fast PIII. Each point is the average of six experiments. Retinas were incubated in the presence of pyruvate throughout the experiment. Solution containing IAA was added at the 30-min mark and removed 20 min later. The same result was obtained when IAA was not removed at the 50-min mark.

Voaden, 1970). This high glycolytic activity seems to be a general property of the mammalian retina.

Reducing the concentration of glucose to 1 mM had no significant effect on the amplitude of Fast PIII but decreased aerobic lactate production by 56%. Under this same condition, retinal aerobic ATP content fell by only 20%. This is consistent with the fact that the Krebs cycle is more efficient than glycolysis in the production of ATP; a limited amount of glucose metabolized aerobically produces significant amounts of ATP. In fact, the levels of ATP in the retina under aerobic conditions were higher than the corresponding anaerobic values for glucose concentrations between 1 and 20 mM. The efficiency of the oxidative pathway was also demonstrated by employing glucose-free media in

the presence and absence of oxygen. Without added glucose, lactate production ceased; electrical activity and ATP content declined; however, the rate of decline was considerably slower in oxygen compared with that in nitrogen. Furthermore, in the glucose-free, oxygenated medium the rates of loss of both Fast PIII and ATP depended upon the concentration of glucose during the control period, indicating greater production of ATP from the residual glucose by aerobic than by anaerobic metabolism.

These results suggest that the oxidative production of ATP for the generation of the electrical activity in the photoreceptor cell in substrate-free medium greatly depends on endogenous stores derived form glucose metabolism. That these stores are predominately carbohydrate is indicated by the findings that the addition of IAA to oxygenated, glucose-free medium accelerated the

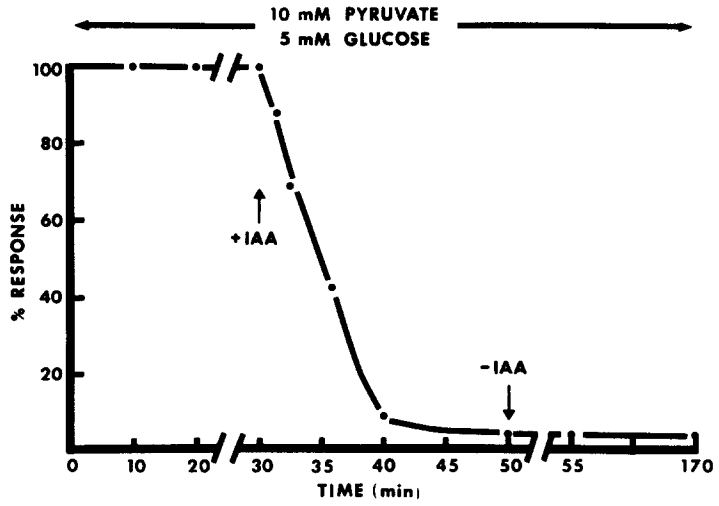

FIGURE 13. The effect of adding IAA (2 mM) for 20 min in the presence of glucose and pyruvate on the relative response amplitude of Fast PIII. Note particularly that Fast PIII disappears under this condition and fails to recover when IAA is withdrawn. Each point is the average of six experiments.

decline in the amplitude of Fast PIII and ATP content and that these changes no longer depended on the control concentration of glucose. The number of hexose equivalents stored in the rat retina during the control period was estimated from the level of anaerobic lactic acid production in the absence of glucose. The magnitude of these equivalents was proportional to the concentration of glucose in the medium. When the control substrate concentration was 20 mM glucose, hexose in the rat retina was highest (200 nmol/mg dry wt). This concentration is lower than that reported for the rabbit retina (Cohen and Noell, 1960); it agrees, however, with data showing that rat retinal glycogen is very low (Kuwabara and Cogan, 1961; Mizuno and Sato, 1975).

It is known that anaerobiosis causes the loss of the *b*-wave and ganglion cell activity in the rat and rabbit retina, as well as loss of Fast PIII in the rabbit (Winkler, 1972, 1975, and unpublished observations). Winkler (1972) also reported that the glycolytic production of ATP was sufficient to maintain the amplitude of Fast PIII in the rat at near normal levels. Hagins et al. (1970)

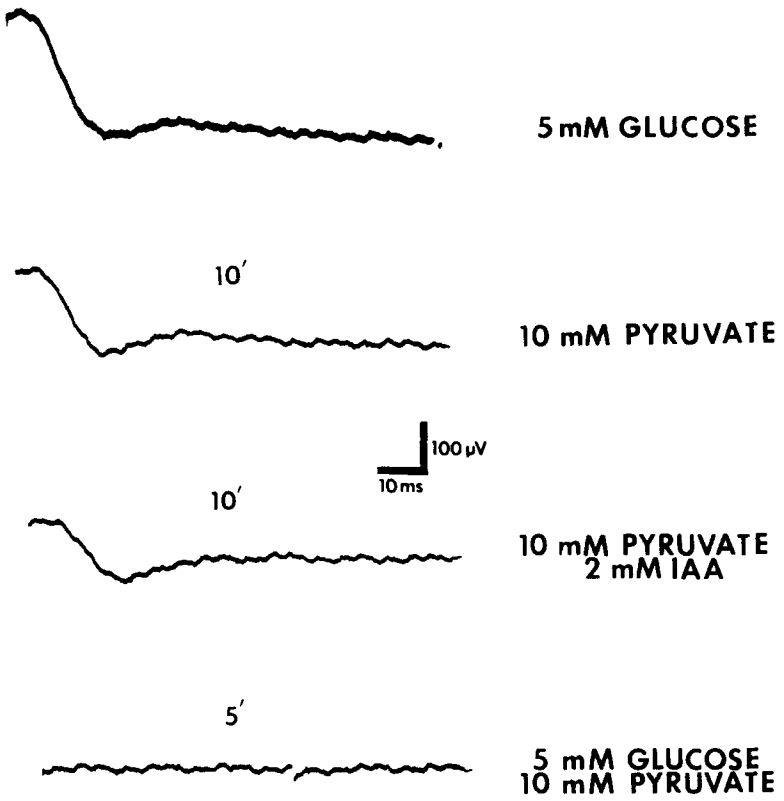

FIGURE 14. Fast PIII tracings from one experiment in which the retina was superfused sequentially with glucose, pyruvate, pyruvate and IAA, and pyruvate and glucose. The superfusion times for each of the test solutions before the light flash is shown above each of the lower three tracings. Although there is a small reduction in the amplitude of Fast PIII when IAA is added to the pyruvate-containing medium, it is clear that there is a rapid loss of this potential when the retina is incubated in the presence of both glucose and pyruvate after treatment with IAA. The light flash coincides with the onset of the trace.

found that this response was exclusively dependent on oxygen. It has been suggested (Winkler et al., 1977) that this difference is related to the type of buffer system used, bicarbonate (Winkler) and Tris-phosphate (Hagins et al.). The present results also support this suggestion, since Fast PIII disappeared when bicarbonate was absent and oxygen was replaced with nitrogen. Fur-

thermore, in bicarbonate-containing medium the extent to which glycolysis alone supported Fast PIII depended on the concentration of glucose. Thus, glycolysis maintained Fast PIII at a high level only when anaerobic lactic acid production was maximal in the presence of 20 mM glucose, the concen-

TABLE VI

RETINAL ATP CONTENT*

Substrate	Absence of IAA	Presence of IAA
	μmol/g dry wt	
5 mM Glucose	9.8±0.9 (5)	2.6±0.5 (11)
10 mM Pyruvate	9.0±0.8 (5)	7.2±0.8 (4)
10 mM Pyruvate with 5 mM glucose	9.5±0.9 (4)	2.6±0.4 (4)
10 mM Pyruvate with 10 mM 2-DOG	3.2±0.4 (5)	3.5±0.5 (3)

* Incubations were for 30 min. Results are expressed as means ± SE. The number of experiments is indicated in parentheses.

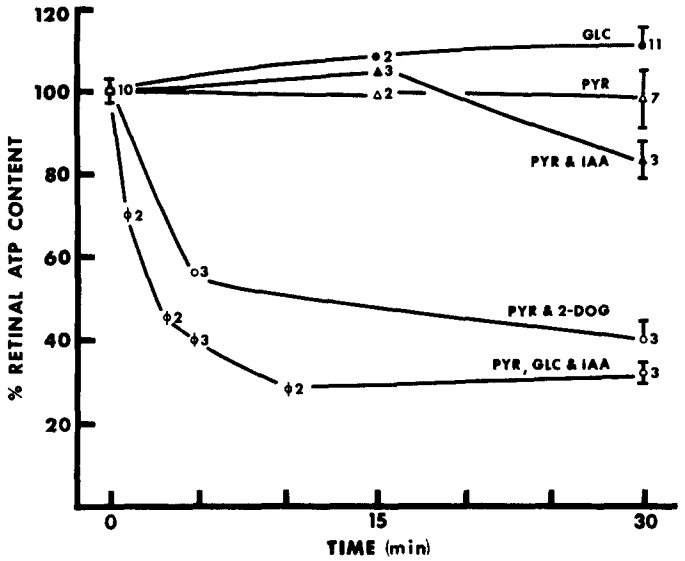

FIGURE 15. A graph of the time-course changes in the relative retinal ATP concentrations as a function of incubation in different combinations of substrates and/or metabolic inhibitors. GLC, glucose; PYR, pyruvate; 2-DOG, 2-deoxyglucose.

tration of glucose used by Winkler (1972) in his earlier study. With 5 mM glucose the amplitude of Fast PIII in the presence of nitrogen declined to ~40% of the control (aerobic) value, indicating that glycolysis alone is no longer capable of maintaining Fast PIII at a high level when a concentration

of glucose similar to that found in the blood of control animals is used. In the presence of 5–20 mM glucose, glycolysis alone is more efficient in maintaining the normal amplitude of Fast PIII and the level of ATP in the retina than are oxidative processes in the absence of glucose. It is clear that both the electrical activity and high-energy content of the retina are extremely dependent upon glucose as a substrate for glycolysis and oxidative metabolism.

The capacity of the superfused rat retina to utilize substrates other than glucose for the production of ATP was determined. As found for brain tissue (Sloviter and Kamimoto, 1970; Maker and Lehrer, 1972), mannose replaced glucose very well in the production of lactate, maintenance of ATP content, and generation of electrical activity in the rat retina. Clearly, mannose entered retinal cells and was phosphorylated by the hexokinase of the retina. This enzyme was also capable of phosphorylating 2-deoxyglucose to its nonmetabolizable product 2-deoxyglucose-6-PO_4, as seen by the rapid depletion of retinal ATP content when this sugar was added to incubation media. The inability of fructose to replace glucose in lactate production appears to be due to a limitation on its entry into retinal cells, since in preliminary experiments with homogenates of whole rat retinas fructose and glucose both produced lactate. This apparent stereospecificity in the transport of sugars into retinal cells suggests that this process is carrier mediated. On the other hand, galactose was unable to produce lactate in the homogenate or intact preparation. Although it is possible that galactose is not transported into retinal cells, it also appears that this tissue does not contain the enzymes necessary for galactose to enter the glycolytic cycle. Furthermore, the addition of 1 mM ATP or other high-energy phosphates to media with or without substrate had no effect in the intact rat retina, suggesting that these compounds also do not readily enter retinal cells.

The results showed that pyruvate (and lactate and glutamine) maintained the amplitude of Fast PIII and the concentration of ATP reasonably well in the absence of glucose, even in the presence of IAA. This indicates that ATP produced by mitochondrial activity is capable of supporting electrical activity when glycolytic production of ATP is blocked. For the photoreceptor cell, it follows that the cellular compartments for glycolysis and respiration feed into the same high-energy phosphate pool that supports the transduction process. Nevertheless, the fact that pyruvate, even at a concentration as high as 20 mM, does not substitute completely for glucose in supporting electrical responsiveness shows that neither glycolysis alone nor Krebs cycle activity alone maintains the retina at an optimal functioning level.

An unexpected result in this study is that when certain hexoses were paired with pyruvate and iodoacetate there was a rapid depletion of ATP and loss of Fast PIII. The hexoses that produced these inhibitory effects were glucose and mannose, the same two sugars that were metabolized under normal conditions. Glucose and mannose, therefore, appear to have a deleterious effect only when their cellular uptake occurs during the blockade of glycolysis by IAA. The results suggest that the "deleterious" effect of glucose on the retina fed pyruvate and IAA may be attributed to a depletion of ATP caused by

hexokinase activity. It is possible that the rate of this ATP-utilizing reaction is faster than the rate of ATP production from the metabolism of pyruvate, a suggestion supported by the finding that 2-deoxyglucose also depletes retinal ATP, even in the presence of pyruvate. It follows that the hexokinase reaction has a high capacity and that glucose phosphorylation by hexokinase is a significant drain on the high-energy sources of retinal cells during IAA-blocked glycolysis. This would seem to be especially true for the photoreceptor cells, since Lowry et al. (1961) have shown that the activity of hexokinase is highest in the inner segments of the photoreceptor cells. It appears, therefore, that the decrease in the amplitude of Fast PIII under this condition is associated with a decline in the concentration of ATP below a critical level that is required to support active ion transport processes or other essential energy-dependent processes.

Clearly, experiments designed to rigorously test whether the inhibition of glycolysis can be bypassed with pyruvate or other intermediates as substrates for respiration should be performed in the absence of glucose after depletion of carbohydrate stores. In a recent paper on the metabolic requirements for active ion transport in the bullfrog cornea Reinach et al. (1977) concluded that pyruvate could not be used as a substrate in the presence of IAA. Interestingly, a high concentration of glucose was present throughout their experiments. It is tempting to suggest that the results and conclusions of Reinach et al, regarding the effectiveness of pyruvate might have been different had they carried out their experiments in glucose-free media.

ATP and GTP are involved in a variety of light-activated enzymatic processes within the outer segment of the photoreceptor cell, including the phosphorylation of rhodopsin (Bownds et al., 1972), hydrolysis of cyclic GMP by phosphodiesterase (Miki et al., 1973; Yee and Liebman, 1978), and a GTPase (Wheeler and Bitensky, 1977; Robinson and Hagins, 1979). It appears that GTP but not ATP, as previously reported (Carretta and Cavaggioni, 1976), is reduced in concentration by the illumination of outer segments (Biernbaum and Bownds, 1979; Robinson and Hagins, 1979). It was anticipated that the light-induced decreases in the level of GTP and cyclic GMP as well as the light-induced suppression of the dark current of photoreceptor cells (Hagins et al., 1970) would be reflected in changes in the glucose metabolism of these cells in darkness and in light. Differentiation of the metabolism of the photoreceptor cells from other cell groups was attempted by studying the effect of light during synaptic blockade with sodium aspartate. Only small differences in aerobic and anaerobic lactate production existed between dark- and light-adapted rat retinas incubated under control conditions and, as measured on the whole retina, ATP levels were not significantly different in darkness and in light. These results are in agreement with previous findings in the rabbit retina that light has little affect on lactate production, glucose oxidation, and respiration (Cohen and Noell, 1960). Jaffee et al. (1975) reported that flickering light (10 flashes/s) caused a small decrease in the oxygen uptake of the whole rat retina, although the maximum rate of respiration in their study was only 3–5% of other published values (Graymore,

1960; Riley and Voaden, 1970). Under anaerobic conditions, light caused a 15–20% increase in the rate of C-1 oxidation of glucose in isolated bovine rod outer segments (Futterman, 1963), which is not inconsistent with the present findings, since the hexose monophosphate pathway accounts for a small fraction of the total glucose utilized and might not be expected to influence substantially the rate of glycolysis.

Metabolic free energy in the form of ATP clearly is required for the operation of various ion transporting systems in the retina, including the sodium-potassium ATPase (Frank and Goldsmith, 1967; Winkler and Riley, 1977), the bicarbonate-or-anion-stimulated ATPase (Winkler and Riley, 1977), and the calcium-stimulated ATPase (Berman, et al., 1977; Winkler and Riley, 1980) systems. It can be argued that a major fraction of ATP production in this tissue is used to support active ionic exchanges that are vital for the maintenance of normal electrical activity. The effects of ouabain support this view and show, moreover, that both glycolysis and respiration provide energy for driving this ATPase. Thus, incubation of the retina with ouabain leads to loss of Fast PIII, presumably due to a breakdown in the sodium concentration gradient and to substantial reductions in both lactate production and respiration (Riley, 1965) but has no effect on retinal ATP content. Ouabain is thus effective in inhibiting the utilization of ATP. Although a bicarbonate-free medium has also been shown to inhibit ATP utilization in the retina (Winkler et al., 1977), it remains to be proved that this inhibition is due to an alteration in the activity of the bicarbonate-stimulated ATPase. Furthermore, there is no information, at present, about the relationship between the activity of the calcium-stimulated ATPase and retinal metabolism. Such studies are necessary for a more complete description of the contributions of different ion transport processes to metabolic activity in the retina.

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