Vol. 168, No. 1, 1990
April 16, 1990

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 155-160

A STAGE IN GLYCOLYSIS CONTROLS THE METABOLIC ADJUSTMENTS OF VERTEBRATE ROD PHOTORECEPTORS UPON ILLUMINATION

Sanford E. Ostroy, Roberta A. Svoboda and Meegan J. Wilson

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907

Received February 13, 1990

The factors affecting the metabolic adjustments of toad rod photoreceptors were studied by monitoring the oxygen utilization of excised retinas and by measuring rod outer segment ATP and GTP concentrations. Respiratory adjustments upon illumination were observed when glucose or fructose was provided in the perfusate, but not when a glycolytic inhibitor was added to the perfusate containing glucose and pyruvate, or when a substrate beyond glycolysis or from a later stage of glycolysis was substituted for glucose. The amplitudes of the respiratory adjustments to illumination were dependent on the concentration of glucose in the perfusate. The ATP and GTP concentration changes were dependent on respiratory adjustments, including glycolytic effects, and on the levels of illumination. The data suggest a control point within glycolysis for light-induced adjustments of respiration, possibly at phosphofructokinase.

INTRODUCTION: Oxidative metabolism provides critical components for the functioning of vertebrate photoreceptors. The major product of oxidative metabolism, ATP, is used for reactions such as primary active transport and the phosphorylation of rhodopsin and other molecules in the photoreceptor. The phosphorylation of GDP produces GTP, the precursor in the synthesis of cGMP (the internal transmitter of the vertebrate photoreceptor). In addition, photoreceptors rely heavily on the pentose phosphate shunt as a major source of NADPH, the nicotinamide coenzyme used in the reduction of the rhodopsin chromophore (1).

Zuckerman and Weiter (2) and Kimble et al. (3) studied the changes in respiration of amphibian (bullfrog) photoreceptors. The usual response to illumination was a decrease in the rate of respiration. Based on the effects of ouabain and external sodium on respiration, both studies concluded that the use of ATP by the Na^+-K^+ ATPase was a major factor affecting the respiration rate of the rod photoreceptors. Kimble et al. (3) also isolated a light-induced stimulation of respiration in the presence of ouabain. He attributed this response to light-induced ATP consuming processes that are usually masked by light-induced reductions in the activity of the Na^+-K^+ ATPase. In the present study we used various substrates and inhibitors to further investigate the factors that affect the metabolic adjustments to illumination of amphibian (toad, Bufo Marinus) rod photoreceptors. We find that a stage in glycolysis, possibly at phosphofructokinase, controls the metabolic adjustments. Abstracts of earlier aspects of this work have been published (4,5).

0006-291X/90 $1.50

METHODS: The methods for measurement of oxygen utilization and ATP concentration were as described previously by Kimble et al. (3). Briefly, the oxygen utilization of an excised retina is monitored with a standard oxygen electrode in a special dual chamber that allows a continuous supply of new perfusate to the retina and the stirring of the solution below the electrode without a loss of photoreceptors. Long term stability of the retina and high sensitivity to small changes of oxygen utilization can be obtained because the system provides a continuous supply of oxygen to the retina and the oxygen concentration is monitored within narrow limits. In the presence of ouabain, illumination-induced changes of respiration can be unequivocally identified with photoreceptors because, without the ionic gradients and membrane potential changes of the photoreceptors, light cannot affect non-photoreceptor cells. In the absence of ouabain, aspartate is used to minimize non-photoreceptor responses. A low light intensity level (below cone threshold) is used in the respiration experiments so that only rod photoreceptors are stimulated. GTP concentrations were determined using the method of Karl (6) in which endogenous ATP is first eliminated and the GTP is then converted to ATP for assay by the luciferin/luciferase technique.

RESULTS: Light-induced changes of respiratory rate were observed only when metabolic substrates used in glycolysis were present in the perfusate. When glucose and pyruvate were both present, light-induced respiratory adjustments were observed, but when the glucose was eliminated from the perfusate the respiratory adjustments were also eliminated. A representative experiment illustrating these data is presented in Fig. 1. Respiratory adjustments to illumination were also eliminated when the glycolytic inhibitor iodoacetic acid (IAA, 2mM, n=6) was added to perfusate containing both glucose and pyruvate. Respiratory adjustments were maintained when fructose was supplied as an alternative glycolytic substrate (5.6 mM, amplitude of 0.32% ±.03, n=10), but not when glycerol (20mM, n=3) or pyruvate (15mM, n=5) were substituted for glucose.

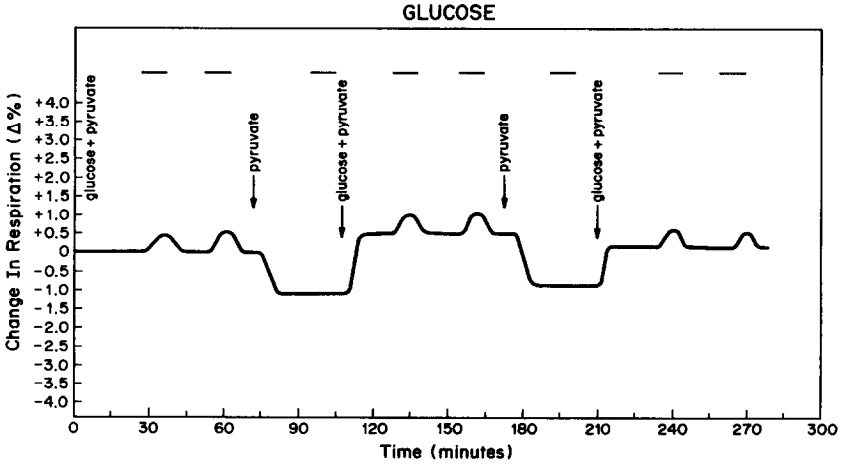

Figure 1. Effect of Metabolic Substrate on Light-Induced Respiratory Responses. Glucose concentration, 5.6mM; pyruvate concentration 15mM. In the presence of ouabain (0.1mM). When both glucose and pyruvate are present in the perfusate light causes a stimulation of respiration. Removing the glucose eliminates the respiratory responses. Representative of 15 similar experiments. Re-addition of glucose restored the respiratory responses in 14 of the 15 experiments. Similar results were obtained in experiments in which the substrate was changed from glucose to pyruvate (n=5). Light intensity was 2.39 X 10^{-7} watts/cm^2 at 500nm, sufficient to bleach approximately 1% of the rhodopsin over a 10 min. period. Similar results were obtained in the absence of ouabain (n=4).

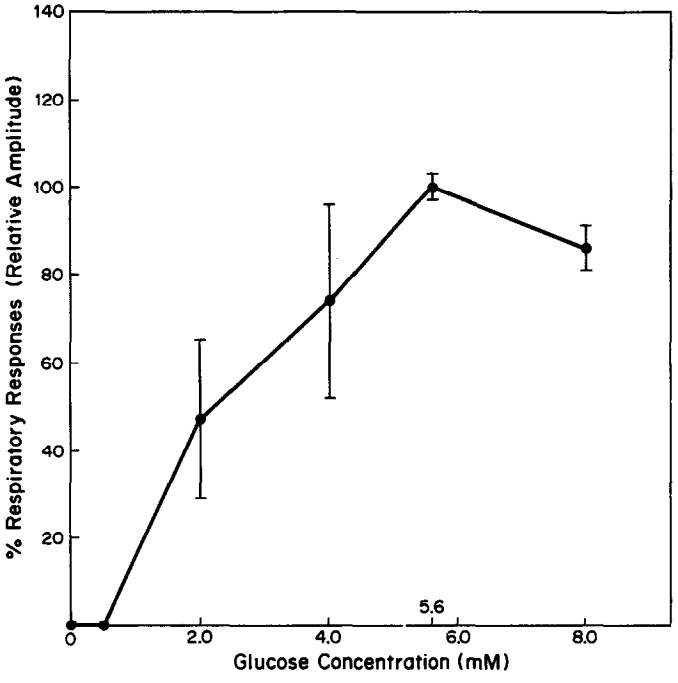

Figure 2. Glucose Dependence of the Amplitude of the Light-Induced Respiratory Responses. Data normalized to 100% at 5.6mM glucose. Amplitude at 5.6mM glucose, 0.36% ±.01 (n=133). Number of determinations as follows: 0mM (n=4); 0.5 mM (n=9); 2.0 mM (n=7); 4.0 mM (n=6); and 8.0 mM (n=7). Experiments performed in the presence of ouabain (0.1mM).

When glucose was the only metabolic substrate supplied in the perfusate, the amplitudes of the light-induced respiratory adjustments were found to depend on glucose concentration. Those data are presented in Fig. 2. No light-induced respiratory responses were observed at 0mM or 0.5mM glucose; increasing respiratory response amplitudes were observed at 2mM, 4mM and 5.6mM glucose, respectively; at 8mM the responses were reduced slightly from the maximal responses observed at 5.6mM.

To evaluate the effect of the respiratory adjustments on high energy nucleotides, the concentrations of ATP and GTP were determined under comparable conditions. The data on ATP and GTP concentrations of rod outer segments in the dark and at five different bleaching levels with various substrates in the perfusate are presented in Fig. 3. With glucose as the substrate, the ATP concentrations did not change significantly from dark levels at bleaches up to and including 1% (Fig. 3A, G). The data show that ATP concentrations are maintained under these conditions. Since these are the conditions under which light-induced decreases of respiration are observed (3), it also indicates that the decreases in ATP production resulting from the decreases in respiratory rate must be off-set by decreases in the utilization of ATP. At bleaching levels of 10% and 50% significant decreases in ATP concentration were observed. The data show that at these high levels of light intensity the photoreceptor is unable to maintain its ATP concentration throughout the illumination period. At these light levels, stimulations of respiration were observed (unpublished observations), suggesting an attempt to off-set

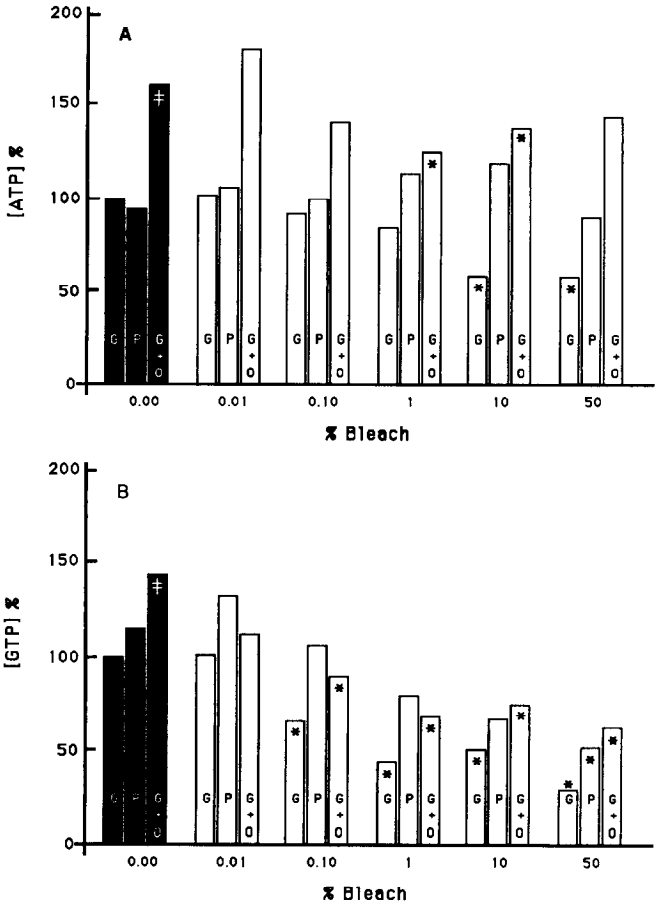

Figure 3. Effect of Substrate and Illumination on the Concentrations of ATP (3A) and GTP (3B). The data were normalized to the dark values with glucose in the perfusate (G, 0.00% bleach, 5.6mM). Absolute values as follows: ATP (1.25 ±0.09 pmole/μg protein); GTP (1.36 ±0.09pmole/μg protein). Other symbols P: pyruvate (15mM); G + O: glucose (5.6mM) plus ouabain (0.1mM); ‡, significant at 99.9% confidence (using the Student's t-test), as compared to glucose in the dark; * significant at >95% confidence level as compared to the same substrate in the dark. For the dark determinations one-half of the retina was maintained in 5.6mM glucose as a control and the other half was maintained in the specified substrate. For each bleaching condition one-half of each retina was maintained in the dark in the same perfusate and the other half was maintained in the light at an intensity level designed to bleach the specified amount at the end of the ten minute illumination period. Glucose dark values present the average of 28 determinations. Each measurement represents 3 to 8 determinations. Unattenuated light intensity was 2.39 X 10^{-5} watts/cm^2 at 500nm.

increases in ATP utilization, but under these conditions the respiratory measurements are not able to unequivocally identify respiratory changes with rod cell responses.

A major feature of the respiratory adjustments was their dependence on glucose and glycolytic substrates (Figs. 1 and 2). To evaluate whether these properties of the photoreceptor could be observed by effects on ATP concentration, pyruvate was substituted for glucose in the perfusate. The data are presented in Fig. 3A (designated as P). The concentration of pyruvate was selected because it provided an ATP concentration comparable to that observed with glucose in the dark and because it had no effect on overall respiratory rates (as compared

to glucose alone, data not shown). On illumination no significant changes of ATP concentration were observed at any bleaching level. At 1% and 10% bleaches slight increases in ATP concentration were even observed. These data are consistent with what one might expect if the light-induced decreases of respiration (and decreases in ATP production) were eliminated under these conditions, but the processes that decrease the utilization of ATP on illumination (presumably a decrease in the activity of the sodium-potassium pump) were not affected in a major way.

In the presence of ouabain the effects of the sodium-potassium pump are eliminated. In the dark a major increase in ATP concentration was observed (as compared to glucose alone, Fig. 3A, G and G+0, 0% bleach) consistent with significant ATP use by the sodium-potassium pump and its elimination in ouabain. In the presence of ouabain, illumination-induced increases in respiration are observed (Fig. 1 and Ref. 3). The light-induced stimulations of respiration would tend to increase the concentration of ATP while the light-induced ATP utilization processes would tend to decrease it. The only significant changes of ATP concentration were decreases, observed at 1% and 10% bleaches.

The GTP data are presented in Fig. 3B. The interdependence of ATP and GTP is illustrated by the dark increase of GTP concentration in the presence of the Na^+-K^+ ATPase inhibitor, ouabain (Fig. 3B, G and G+0, 0% bleach). GTP concentration changes exhibit some properties in common with ATP concentration changes. Thus, on elimination of the light-induced decreases of respiration by substitution of pyruvate for glucose in the perfusate, significant light-induced decreases of GTP were only observed at one bleaching level (50%), whereas in glucose, significant decreases in GTP occurred at four bleaching levels (0.1%, 1%, 10% and 50%). The GTP concentration appeared more sensitive to illumination than ATP (which exhibited light-induced concentration changes at two bleaching levels in glucose and none in pyruvate). The data illustrate an inability of the metabolic reactions to off-set the GTP concentration changes within the time-course of the illumination at bleaching levels of 0.1% and higher. The sensitivity of GTP to illumination is not unexpected considering the number of GTP related reactions that are associated with phototransduction and adaptation (reviewed by Ref. 7).

DISCUSSION: A number of the experiments demonstrate the importance of glycolysis to the metabolic adjustments of rod photoreceptors upon illumination. These results include: the dependence of the respiratory response amplitudes on glucose concentration in the perfusate (Fig. 2); the elimination of respiratory responses when the glycolytic inhibitor, IAA, is added to glucose/pyruvate perfusate; and the absence of respiratory responses when pyruvate, a substrate that enters the metabolic pathway subsequent to glycolysis, is substituted for glucose (Fig. 1).

The data suggest that the control step for these adjustments to illumination is between fructose-6-phosphate and glyceraldehyde-3-phosphate. The presence of respiratory responses when one provides glucose or fructose (which may enter the glycolytic pathway at fructose-6-phosphate) but absence of responses with glycerol (which may enter the pathway at glyceraldehyde-3-phosphate) or pyruvate are the pertinent data. It is noteworthy that the

conversion of fructose-6-phosphate to fructose-1-6-bisphosphate (one of the two reactions within the suggested control point) is catalyzed by phosphofructokinase, a well documented controlling enzyme within glycolysis (8). However, with the presently available data the responsible reaction(s) cannot be unequivocally identified because it is not certain that the glycerol is being metabolized.

Overall, the data are consistent with the following view of the metabolically related processes of the rod photoreceptor. At low levels of illumination, reduced use of ATP by the Na^+-K^+ ATPase and a feedback of the increased ATP (or reduced ADP) on phosphofructokinase, cause a reduction in the rate of respiration. ATP is being used by the photoreceptor for light-induced reactions, but this use is more than off-set by the ATP available from the reduced activity of the Na^+-K^+ ATPase. At the lowest levels of illumination GTP concentration is also maintained (bleach of 0.01% over a 10 min. period of illumination). However, at higher intensities the light-induced reactions that utilize GTP become more prominant, while the reactions that restore GTP (mainly the phosphorylation of GDP by ATP) do not increase in rate sufficiently, so that the GTP concentration is reduced, even at relatively low bleaching levels (bleaches of 0.1% and 1% over a 10 min. period of illumination). At intensity levels sufficient to close all of the sodium channels, reduced ATP usage by the Na^+-K^+ ATPase is no longer a variable and the use of ATP and GTP by the light-induced processes of the photoreceptor now dominate. Although respiration may be stimulated under these conditions, it is apparently insufficient to maintain either ATP or GTP concentrations throughout the period of illumination.

These studies show that extracellular levels of glucose, or a reduction in glycolysis, or high bleaching levels may affect the ability of the respiratory system to maintain the photoreceptor's concentration of ATP and GTP. It would therefore not be surprising if these conditions also adversely affected the maintenance or functioning of the photoreceptors (9).

ACKNOWLEDGMENTS: We thank Brenda Putman for performing certain experiments. We thank Michael Allen, Anise Ardelt, Joanne Cusumano and Clark Gedney for important help with the manuscript. This work was supported by Public Health Service Grant EY00413.

REFERENCES

1. Futterman, S. (1963) J. Biol. Chem. 238, 1145-1150.
2. Zuckerman, R., and Weiter, J.J. (1980) Exp. Eye Res. 30, 117-127.
3. Kimble, E.A., Svoboda, R.A., and Ostroy, S.E. (1980) Exp. Eye Res. 31, 271-288.
4. Ostroy, S.E., Svoboda, R.A., Meyertholen, E.P., and Wilson, M.J. (1982) Fed. Proc. 41, 750.
5. Ostroy, S.E., Meyertholen, E.P., Wilson, M.J., and Svoboda, R.A. (1982) Invest. Ophthal. Vis. Sci. 22, 229.
6. Karl, D. (1978) In Methods in Enzymology (S.P Colowick and N.O. Kaplan Eds.; M.A. DeLuca, Vol. Ed.) Vol. LVII, 85-94. Academic Press, New York.
7. Yau, K.-W., and Baylor, D.A. (1989) Ann. Rev. Neurosci. 12, 289-327.
8. Smith, E.L., Hill, R.L., Lehman, I.R., Lefkowitz, R.J., Handler, P., and White, A. (1989) Principles of Biochemistry: General Aspects. pp 395-398. McGraw Hill, New York.
9. Baker, B.N., and Willliams, T.P. (eds.). (1980) The Effects of Constant Light on Visual Processes. Plenum Press, New York, 455 pp.