The Light-induced Increase of Carbohydrate Metabolism in Glial Cells of the Honeybee Retina Is Not Mediated by K⁺ Movement Nor by cAMP

V. Evêquoz-Mercier and M. Tsacopoulos

From the Experimental Ophthalmology Laboratory, Department of Otoneuro-Ophthalmology, University of Geneva Medical School, CH-1211 Geneva 4, Switzerland

ABSTRACT The retina of the honeybee drone is a nervous tissue in which glial cells and photoreceptor neurons constitute two distinct metabolic compartments. The phosphorylation of glucose and its subsequent incorporation into glycogen occur essentially in glia, whereas O₂ consumption occurs in the photoreceptors. After [³H]glucose loading of superfused retinal slices, light stimulation induced a significant rise in [³H]glycogen turnover in the glia. This occurs without a concomitant covalent modification of glycogen enzymes. Probably only an increase or a decrease of the availability of [³H]glycosyls that are incorporated into glycogen is necessary. As only photoreceptors are directly excitable by light, we searched for a signal that stimulates glycogen metabolism in the glia. Although K⁺ in extracellular space and glia increases after repetitive light stimulation, increasing bath K⁺ in the dark did not mimic the metabolic effects of light, despite an equivalent increase of K⁺ in the extracellular space and glia. We subsequently explored the role of cAMP, a universal intracellular second messenger. Exposure of retinal slices to the adenylate-cyclase activator forskolin induced an expected increase in the rate of formation of cAMP, but only partially mimicked the metabolic effects of light. Furthermore, light stimulation failed to induce a rise in the rate of formation of cAMP. We conclude that in this nervous system, without synapses, neither K⁺ nor cAMP mediates the effect of light stimulation on intraglial glucose metabolism.

INTRODUCTION

Nervous tissues are made of neurons and glial cells. The first thorough and direct study on the physiological properties of the glial cell was made by Kuffler and collaborators on the nervous system of the leech and of the mudpuppy (see Kuffler and Nicholls, 1976). The essential information provided by these studies was that the membrane of the glial cells behaves passively in response to the stimulation of neurons as a "perfect K⁺ electrode" (Kuffler, Nicholls, and Orkand, 1966). Since neurons and glial cells are separated from each other by extracellular space, Kuffler...
and Nicholls (1966) hypothesized that neurons transmit signals to glial cells by releasing K⁺ into the extracellular space during excitation. Coles and Tsacopoulos (1979) were the first to measure K⁺ activity with microelectrodes in the three compartments of nervous tissue (i.e., neuron, glial cell, and extracellular space). Their measurements confirmed to some extent the above hypothesis by showing that the stimulated neurons (the photoreceptors of the honeybee drone retina) release K⁺ ions that transiently accumulate in the extracellular space and depolarize the membrane of the glial cells. In the course of these studies two important and related questions were raised. The first was do glial cells respond metabolically to potassium-mediated depolarization and does their metabolism play a role in supplying nutritive material to neurons? Increased extracellular K⁺ (Kₑ) does indeed affect carbohydrate metabolism in both glia and neurons of the isolated ganglia of the leech and mouse cerebral cortex (Pentreath and Kai-Kai, 1982; Hof, Pascale, and Magistretti, 1988). However, these results appear complex since these nervous tissues contain synapses and it is conceivable that elevated K⁺ causes the release of neurotransmitters that, in turn, induce glycogen hydrolysis. Since the drone retina has no synapses (see Perrelet, 1970), we are concerned only with a direct effect. In addition, the superfused slices of drone retina appear to be the adequate model for exploring the role of K⁺ in the metabolic interactions between neurons and glial cells for two reasons. First, the investigation of ionic interactions between neurons and glia, and more particularly of K⁺ homeostasis, has been more direct and carried further in the drone retina than in vertebrate nervous system preparations (Coles and Tsacopoulos, 1979; Coles and Orkand, 1983; for review, see Coles, 1989). Second, the separation of metabolic functions between glial cells and photoreceptors is complete: photoreceptors contain large numbers of mitochondria and have a very active aerobic energy metabolism (Tsacopoulos and Poitry, 1982; Dimitracos and Tsacopoulos, 1985; Jones and Tsacopoulos, 1987); in contrast, glial cells contain > 250 times fewer mitochondria than the photoreceptors (see Fig. 1 in Dimitracos and Tsacopoulos, 1985; Tsacopoulos, Coles, and Van de Werve, 1987) but large quantities of glycogen β particles (Perrelet, 1970; Tsacopoulos et al., 1987). Photoreceptors do not contain glycogen; it is undetectable in electron micrographs (Perrelet, 1970; Baumann, 1974; Coles and Tsacopoulos, 1981; Baumann and Walz, 1989; Coles, 1989) and histochemical staining is negative (Tsacopoulos et al., 1987). Experimental evidence suggests that glucose itself is not a substrate for the photoreceptors: first, 3H-2-deoxyglucose (H-2DG) autoradiography and biochemistry show that the phosphorylation of this glucose analogue, irrespective of intense light stimulation or darkness, occurs predominantly in glial cells. Under these experimental conditions, radioactivity of 3H-2DG-6P in photoreceptors was not higher than the extracellular background radioactivity (Tsacopoulos, Evéquoz-Mercier, Perrottet, and Buchner, 1988; Braziti-kos and Tsacopoulos, manuscript submitted for publication). Second, superfusion of isolated drone retinas with Ringer carrying high levels of 2-DG does not affect oxygen consumption by photoreceptors, as one would expect if hexokinase were the first enzyme in photoreceptor metabolism (Tsacopoulos et al., 1987). Thus, based on these arguments we have concluded that in superfused slices glycogen metabolism and its stimulation by light also occur predominately in the glial cells (Tsacopoulos et al., 1987; Tsacopoulos et al., 1988).
METHODS

Preparation of Slices

Retinal slices (250–300 μm thick) were prepared as described in detail by Tsacopoulos et al. (1987). Briefly, two parallel cuts with a vibrating razor blade were made parallel to the ommatidia in the dorsal region of the retina. Slices incubated at room temperature in Ringer solution equilibrated with 100% O₂ (containing 270 mM NaCl, 10 mM KCl, 10 mM MgCl₂, 1.6 mM CaCl₂, 10 mM Tris buffer at pH 7.4), were well oxygenated throughout (Tsacopoulos, Poitry, and Borsellino, 1981) (Fig. 1). The viability of the preparation was checked by measuring with O₂-sensitive microelectrodes the decrease in partial pressure of O₂ in the tissue induced by intense light stimulation during superfusion (see Tsacopoulos and Poitry, 1982).

Uptake of [3H]Glucose and Incorporation into Glycogen

Slices were incubated in the dark for 15, 30, 45, 60, and 90 min in oxygenated Ringer solution carrying 24 μM of glucose (400 μCi/ml of [U-14C]glucose; New England Nuclear, 15.7 Ci/mmol). After each period of incubation, the total radioactivity in the homogenate of the retina and the radioactivity incorporated into glycogen were measured. The radioactivity incorporated into glycogen increased roughly linearly and in parallel with the total radioactivity for up to 90 min of incubation (Fig. 2 B). There was no sign of saturation for incubation times up to 60 min. After this time of incubation the average radioactivity was 1.14 ± 0.07 \times 10^6 cpm/mg dry weight (dw) (mean ± SEM, n = 20). A small fraction (2.24 ± 0.26 × 10^5 cpm/mg dw, i.e., ~2%) was incorporated into glycogen. For the experimental procedure we chose to incubate the slices for 60 min.

Experimental Procedure

Retinal slices were maintained in darkness and loaded by incubation (60 min) in Ringer solution carrying [U-14C]glucose (Fig. 1 A). Slices were then rinsed for ~30 s in substrate-free Ringer solution to remove [14C]glucose adhering to the surface. In most experiments the two retinas in a given slice were separated (Fig. 1 B): one was frozen immediately and used as a control, whereas the second was placed in a chamber and superfused with fully oxygenated glucose-free Ringer solution either in the dark or under stimulation by light flashes or
Continuous light. As described in Results, in some experiments the superfusate contained either 20 mM of K⁺ and 260 mM of Na⁺, or 50 μM dinitrophenol (DNP) (Fluka Chemie, Buchs, Switzerland), or 10⁻⁴ M forskolin (Sigma Chemical Co., St. Louis, MO). After 15, 30, or 60 min of superfusion the second retina was also frozen in a chilled propane-isopentane mixture, lyophilized, dissected from the brain (see Tsacopoulos et al., 1987), weighed (~120 μg per retina), and homogenized by sonication in 300 μl of 1M HClO₄. The total radioactivity was

Figure 2. (A) HPLC chromatograms of retinal homogenates. Since total radioactivity after 60 min of incubation is much higher than after 15 min (see curve in B), the 60-min sample was diluted in order to match maximum radioactivity to ~3,000 cpm. Thus the comparison of the two chromatograms is only qualitative. Trh, trehalose. (B) Total radioactivity (●) and radioactivity incorporated into glycogen (○) as a function of time of incubation. Each point corresponds to a different retina.
measured in aliquots of homogenate by liquid scintillation counting. The label incorporated into glycogen was measured following a specific extraction described elsewhere (Evêquoz, Stadelmann, and Tsacopoulos, 1983). The results were expressed as the ratio between cpm·mg⁻¹ dw in the experimental retina and cpm·mg⁻¹ dw in the control retina.

**High Pressure Liquid Chromatography (HPLC)**

Deproteinated homogenates of the retina were analyzed by HPLC on an anionic-exchange column (Aminex HPX-87H; Bio-Rad Laboratories, Richmond, CA) at 22°C and at flow rate of 0.3 ml/min using a 420 Kontron pump. The elution solvent was 0.01 N H₂SO₄ or H₂O. Fractions were collected every 30 s. For identification of the peaks, standard solutions of ¹⁴C-labeled glucose, glucose-6P, glycerol-3P, lactate, and pyruvate (New England Nuclear, Boston, MA) were used. We mention here briefly the basic experiment that lead to the identification of the [³H]trehalose peak. This is important because in a previous paper (Tsacopoulos et al., 1988) we made an erroneous interpretation of the chromatograms; the HPLC method used then in our laboratory did not allow a good separation of the phosphorylated products of glucose. As shown in Results, the separation is now excellent and several lines of evidence strongly suggest that the peak eluted between glucose-6P and glucose (both identified) corresponds to trehalose. The fractions corresponding to the suspected trehalose peak were collected, frozen, and lyophilized. The residue was dissolved in water and incubated 60 min at 37°C with a solution containing the enzyme trehalase, which degrades specifically trehalose to 2 molecules of glucose, and was analyzed again by HPLC. This time the peak appeared at the position of glucose as expected if the collected fractions contained only trehalose.

**Enzyme Assays**

For the determination of enzyme activity the retinas were homogenized in a glycyl-glycine buffer (10 mM, pH 7.4) containing 0.1 M NaF, 10 mM EDTA, 0.5% glycogen, and 0.005% Triton X-100.

**Phosphorylase a activity** was determined as described by Van de Werve, Van den Berghe, and Hers (1974) with some modifications. Briefly, 40 μl of the retinal homogenates was mixed with 40 μl of reagent containing 0.1 M glucose 1-P (pH 6.1), 2% glycogen, 0.2 M NaF, and 1 mM caffeine and incubated for 60 min at 30°C. The reaction was stopped by the addition of 0.2 ml of 10% cold trichloroacetic acid, and the release of inorganic phosphate was subsequently measured by addition of a mixture of 1 ml H₂O, 0.2 ml H₂SO₄-molybdate, and 0.08 ml of aminonaphtholsulfamic reagent. The absorbance was read at 660 nm 15 min later. 1 U of phosphorylase a is the amount of enzyme that liberates 1 μmol of inorganic phosphate per minute at 30°C.

**Synthetase a and total synthetase** were determined as described by Thomas, Schlender, and Larner (1968), and De Wulf and Hers (1968). Briefly, 20 or 30 μl of retinal homogenates was incubated at 30°C with 180 μl of reagent containing glycyl-glycine 0.05 M (pH 7.4), UDPG 1 mM, UDPG-¹⁴C (~200,000 cpm/ml), 1% glycogen, 1 mM EDTA, 10 mM Na₂SO₄ for synthetase a determination or with 180 μl of reagent containing Tris HCl 0.1 M (pH 7.8), 0.5% glycogen, EDTA 4 mM, glucose-6P 5 mM, UDPG 5 mM, UDPG-¹⁴C (~200,000 cpm/ml) for the determination of the total amount of synthetase. After 60 min of incubation, 170 μl was spotted on Whatman paper ET 31 and plunged immediately into cold 66% ethanol. Then the papers were washed three times in cold 66% ethanol, then washed in acetone, dipped in diethylether, and dried before counting by liquid scintillation.
**cAMP Determination**

We used a modified version of the [³H]adenine method first described by Shimizu, Daly and Creveling (1969). Briefly, this method consisted of incubating a slice of retina maintained in the dark in Ringer containing 400 μCi/ml of (8)[³H]adenine (~17.4 μM, specific activity 23 Ci/mmol, Amersham Corp., Arlington Heights, IL) for 60 min. Thereafter, the slice was rinsed and then superfused for 15 min with oxygenated Ringer solution containing 100 μM 3-isobutyl-1-methylxanthine (IBMX) under various experimental conditions: darkness, light flashes, addition of forskolin, or DNP. At the end of this period the slice was frozen, lyophilized, dissected, homogenized by sonication in 300 μl TCA 5%, centrifuged, and extracted five times by diethyl ether saturated with H₂O in order to remove TCA. Four slices per experimental condition were pooled together in order to increase the volume of the tissue sample. The extracts were lyophilized, and then resuspended in 100 μl H₂O and analyzed by reverse-phase HPLC at 22°C using a Li-Chrosorb RP-18 5 μm column (Merck & Co., Rahway, NJ). The flow rate was 1 ml/min and the elution solvent was a mixture of Na₂HPO₄ 0.05 M, tetrabutylammonium phosphate 0.005 M in PIC A reagent from Waters Associates, Milford, MA (pH 6.8).

**Light Stimulation**

The superfused slice was stimulated either with intense 50-ms light flashes presented to the retina every 4 s or continuous light. The optical arrangement and calibration of light stimuli have been described in detail in previously published papers (Tsacopoulos and Poitry, 1982; Jones and Tsacopoulos, 1987).

**Microelectrodes and Calculation of the Oxygen Consumption (QO₂)**

The techniques of making O₂-sensitive microelectrodes and calculating QO₂ from PO₂ changes in a superfused slice have been described in Tsacopoulos et al. (1981) and Tsacopoulos and Poitry (1982). The [K⁺] measurements were made as described by Coles and Tsacopoulos (1979).

**RESULTS**

**HPLC Analysis of the Homogenates after Incubation in Ringer Carrying [³H]Glucose**

Fig. 2A shows the chromatogram of two samples of homogenate: one was made after 15 min of incubation and the other after 60 min. Most of the radioactivity in the retina was in nonphosphorylated glucose and glycolytic products. The distribution among the different peaks changed as a function of time of incubation. For example, after 15 min of incubation a large proportion of the total radioactivity in the homogenate was still in glucose. However, after 60 min of incubation the major part of the radioactivity was in the disaccharide trehalose. As mentioned in Methods, ~2% was incorporated in glycogen. In the experiments that we describe below, the incubation time was 60 min.

**Effect of Light Stimulation on Incorporation of [³H]Glucose into Glycogen**

Retinas loaded with [³H]glucose were transferred to a different chamber and superfused with glucose-free Ringer solution. Under these conditions, light stimulation with intense light flashes for 15 min induced an increase in the incorporation of


$[^3]H$glycosyls into glycogen (Fig. 3A). Stimulation with continuous light for 15 min had a similar effect (Fig. 3A). Measurements of the activity of enzymes of glycogen metabolism in dark-adapted retinas and in retinas receiving 15 or 60 min of light stimulation showed no covalent modification of either phosphorylase or synthetase induced by light stimulation (Fig. 3B and Table I). Stimulation of the retina for longer periods (30 and 60 min) caused a drop of $[^3]H$glycosyls incorporated into glycogen (Fig. 3A). After 60 min of stimulation, radioactive glycogen in the stimulated retinas was significantly less than in the control retinas. Since phosphorylase was not covalently modified (Fig. 3B), this decrease was probably due to the prolonged stimulation of glycolysis leading to a faster decrease of the available

![Graph A: Effect of light stimulation on the radioactivity in glycogen.](image)

**Figure 3.** (A) Effect of light stimulation on the radioactivity in glycogen. The number of slices in each individual column was between 11 and 32 (60 min in dark). Error bars are SEM. The difference between dark and light (15 and 60 min) is statistically significant ($P < 0.0001$ and $P < 0.001$). (B) Activity of phosphorylase $\alpha$ as a function of superfusion with substrate-free oxygenated Ringer either in the dark (●) or while stimulated with light flashes (○). Zero time corresponds to slices frozen immediately after dissection. Arrows indicate the value measured in slices exposed 30 min to Ringer containing 20 mM of glucose; the difference is statistically significant. Each point is the average value from 12 slices distributed in three equal batches and homogenized together. Error bars are SEM. This experiment was made with Dr. G. Van de Werve in January 1985. The experiment dealing with the effect of glucose was repeated twice during autumn 1989 and provided similar results. (C) HPLC chromatograms of homogenates of retinal slices incubated for 60 min with $[^3]C\text{(U)}$-glucose (304.7 mCi/mmol, 330 μM of glucose). In the control, the slice was immediately frozen after incubation, whereas in the two other experiments the slice was superfused for 20 min with substrate-free oxygenated Ringer, either in the dark or while stimulated with light flashes.
[\textsuperscript{3}H]glucose, mainly provided by intracellular [\textsuperscript{3}H]trehalose (Fig. 3 C). Such a subtle activation of glycogen enzymes by end product levels of glycolysis would be undetectable by the method used, but should lead to the mobilization of glycogen stores and therefore to the decrease of [\textsuperscript{3}H]glycosyls incorporated into glycogen. When [\textsuperscript{3}H]glucose was present in the extracellular space and in the cells during superfusion (60–80 min), this light-induced decrease did not occur (data not shown; see also Evêqueoz et al., 1983).

When retinas were superfused for 15 min in the dark with glucose-free Ringer solution, there was, in contrast to the results obtained with light stimulation, a decrease of ~40% of [\textsuperscript{3}H]glycosyls incorporated into glycogen (Fig. 3 A). Longer superfusions (30 and 60 min) caused little additional drop in the radioactivity incorporated into glycogen. Because the labeled glycogen was a very small fraction of the total glycogen, the measurements were not precise enough to allow an accurate determination of the curve describing the decrease of [\textsuperscript{3}H]glycogen as a function of time of superfusion in the dark. Extrapolating from the biochemistry of the mammalian liver, we initially thought that this drop was due to the activation of phosphorylase \( a \) when the retina was transferred from the incubation medium containing 24 \( \mu \text{M} \) of glucose to the glucose-free Ringer solution. The activity of phosphorylase \( a \) was 20–30% lower than in glucose-free solution when the retina was superfused for 30 min with Ringer containing 20 mM glucose (Fig. 3 B). However, with 1 mM glucose in the superfusate (i.e., still 40 times more than in the incubating solution), a covalent modification of phosphorylase \( a \) had not detectably decreased. This result is difficult to reconcile with the above formulated hypothesis. We think rather that superfusion with glucose-free Ringer exerts a “pull” effect on [\textsuperscript{3}H]glycosyls (see Discussion). Radioactive glycosyls are the ones at the ends of the glycogen and the first to be removed when glucose concentration is decreased (Devos and Hers, 1979), so a small change in total glycogen leads to a disproportionate reduction in radioactive units. In another experimental condition (45-min incubation in Ringer containing 80 mM of glucose and 250 \( \mu \text{M} \) of [\textsuperscript{3}H]glucose) the retina still contained large amounts of nonphosphorylated glucose for a long period of

| Experiment   | Phosphorylase \( a \) U/g dw ± SD | Synthase \( a \) mU/g dw ± SD | Synthase \( a + b \) mU/g dw ± SD |
|--------------|----------------------------------|-------------------------------|----------------------------------|
| Control      | 9.26 ± 4.31                      | 12.8 ± 2.90                   | 1727 ± 516                       |
| Dark         | 10.16 ± 4.98                     | 9.08 ± 1.29                   | 1954 ± 598                       |
| Light        | 7.86 ± 4.03                      | 13.2 ± 1.51                   | 1929 ± 687                       |
| DNP 50 \( \mu \text{M} \) | 6.90 ± 2.59                     | 13.88 ± 5.40                  | 1834 ± 410                       |
| Forskolin 100 \( \mu \text{M} \) | 6.93 ± 1.34                     | 14.86 ± 4.39                  | 2210 ± 172                       |
superfusion with glucose-free Ringer solution. In this experimental case there was no
decrease of the radioactivity incorporated into glycogen after 30 and 60 min of
superfusion, but rather a small increase (results not published).

The results depicted in Fig. 3 clearly show that photostimulation of the retina
induces a significant rise of glycogen turnover. Light stimulates only photoreceptors
(Muri and Jones, 1983; Jones and Tsacopoulos, 1987) and the absorption of light by
screening pigments in the pigmented glial cells does not induce electrical signals
(Coles and Schneider-Picard, 1989) nor increase of glycogen turnover (Evéquoz et al.,
1983). Furthermore, in the present work, stimulation of slices loaded with [3H]glu-
cose with light intensities below the threshold of inducing a measurable ΔQO₂ in
response to individual flashes (about I/I₀ = −3.6 log; see also Tsacopoulos and
Poity, 1982) did not cause any change in radioactive glycogen relative to dark-
adapted slices. Since the phosphorylation of glucose and glycogen metabolism most
likely occur in the glia the following question was of interest: what is the signal
between the phototransformed rhodopsin in the photoreceptors and glucose metab-
olism in the glia?

**K⁺ Movement as Metabolic Signal**

The stimulus-induced changes of K⁺ activity in the drone retina have been studied in
detail in our laboratory using K⁺-sensitive microelectrodes (see Coles and Tsacopou-
los, 1979; Coles and Orkand, 1983; Coles and Schneider-Picard, 1989). During
repetitive stimulation with light flashes for 90 s there is a net increase of intraglial
[K⁺] of ~ 10 mM, which is a small relative increase since the intraglial [K⁺] is ~ 132
mM (Coles, Orkand, Yamate, and Tsacopoulos, 1986). An experimental increase in
bath [K⁺] from 7.5 to 18 mM causes a depolarization of the glial membrane and an
increase in intraglial [K⁺] in the dark as large as that induced by repetitive light
stimulation of the photoreceptors (Coles and Orkand, 1983). This uptake of K⁺ by
glial cells explains why during exposure of a superfused slice to high K⁺ for ~20
min, [K⁺], rises slowly and does not reach the value applied in the bathing solution
(see Coles and Schneider-Picard, 1989).

We explored the metabolic effects of bath K⁺ in the dark, using the same protocol
as in the experiments with K⁺-sensitive microelectrodes. Increasing [K⁺] in the bath
from 10 mM (about the normal concentration in the extracellular space) to 20 mM
had in the dark a negligible effect on the QO₂ of photoreceptors. A small and slow
increase of QO₂ was recorded when [K⁺] in the bath was higher than 40 mM. A
representative recording is shown in Fig. 4 and compared with the ΔQO₂ induced by
repetitive stimulation of the same retina with light flashes: ΔQO₂ caused by superfu-
sion of the retina with 50 mM [K⁺] is ~ 1/25 of that induced by repetitive stimulation.
The small increase of QO₂ is possibly due to a small rise in the intracellular
concentration of Ca²⁺ in the photoreceptors as a consequence of membrane depolar-
ization (for the role of Ca⁺, see Fein and Tsacopoulos, 1988).

To test the effect of raising K⁺ on glycogen turnover, we used 20 mM K⁺, which
causes intraglial [K⁺] to rise as much as during repetitive light stimulation (see Coles
and Orkand, 1983), and produces maximal increase in ³H-2DG uptake and incorpo-
ration into glycogen (Pentreath and Kai-Kai, 1982). Additionally, as 20 mM of K⁺ has
very little effect on the QO₂ of the photoreceptors, it could in principle be used to
separate the effect of \( \text{QO}_2 \) on glycogen turnover from the direct effect of \( \text{K}^+ \) on glycogen. The results presented in Fig. 5 show that raising \([\text{K}^+]_o\) in the dark from 10 to 20 mM had a negligible effect on the intraglial glycogen turnover.

We have already shown that exposure of retinal slices to 50 \( \mu \text{M} \) DNP caused a rise of \( \text{QO}_2 \) in the dark equivalent to that caused by repetitive light stimulation (see Fig. 6 of Tsacopoulos et al., 1987) without affecting \([\text{K}^+]_o\) (not shown). In Fig. 5 we present the effect of 50 \( \mu \text{M} \) DNP on \[^{[\text{H}]}\text{glycogen}\). The effect is similar to that induced by light: after a transient rise in radioactivity incorporated into glycogen, there is a significant decrease (relative to the dark) after 60 min of exposure. During long exposures to 50 \( \mu \text{M} \) DNP (Fig. 6), \( \text{PO}_2 \) measured locally in the retina tended to recover towards the baseline but did not reach it after 60 min (longest exposure in these experiments). This indicates that \( \text{QO}_2 \) during the effect of DNP is much higher than in the control (see Tsacopoulos et al., 1981). Thus, an increase of \( \text{QO}_2 \) of the
photoreceptors in the dark can be accompanied by a change of intraglial glycogen turnover in the absence of elevated extracellular [K⁺]. We show below that exposure to 50 μM of DNP induces a rise in cAMP.

The Role of cAMP as Metabolic Signal

Another obvious candidate for such a signal was the release in the extracellular space by the depolarized photoreceptors of an amino acid or another chemical (see Discussion) that could in turn induce a rise of cAMP in the glial cells. The HPLC chromatogram presented in Fig. 7A shows that adenine, adenosine, ATP, and cAMP are well separated. The chromatogram presented in Fig. 7B was obtained from the homogenate of four retinas that had been incubated in the dark for 60 min in the presence of [³H]adenine and then superfused in the dark for 15 min with oxygenated...
Ringer carrying 100 μM IBMX. A large part of the [3H]adenine that entered the cells was transformed into [3H]ATP and into [3H]adenosine (and/or [3H]ADP). Only a small portion of [3H]ATP was transformed to [3H]cAMP (~0.22%), so that it was necessary to use a much more sensitive scale after the elution of [3H]ATP in order to see the peak of [3H]cAMP appearing at an elution time of ~30 min.

Repetitive stimulation of the retina with light flashes for 15 min (starting immediately after incubation) caused a 50% decrease of the content of all labeled adenines, probably because the turnover of adenosines is greatly increased by such a stimulation. There was also a decrease in the amount of [3H]cAMP. However, as in the retinas maintained in the dark, radioactivity in [3H]cAMP was 0.2% of the radioactivity in [3H]ATP. Consequently, this long-lasting stimulation did not cause a net rise in the formation of labeled cAMP in the drone retina (Fig. 7 C). We could have missed a transient rise of cAMP induced by light, so we did experiments in which the retina was stimulated for much shorter periods, either 4 or 47 flashes presented to the retina every 1.3 s. About 50 ms after the last flash the retina was shock-frozen.

Chromatograms of dark-adapted retinas and stimulated retinas (for ~5 or 60 s) showed no difference in [3H]ATP and [3H]cAMP (Fig. 7 D). In contrast, exposure of the retina for 15 min to 100 μM forskolin induced nearly a sixfold rise of [3H]cAMP without affecting significantly the amount of radioactivity incorporated in ATP (Fig. 7 E). To a lesser extent, exposure to 50 μM DNP caused a threefold increase in the formation of [3H]cAMP (Fig. 7 F). However, note that because of the uncoupling effect of DNP on mitochondria, less [3H]ATP is formed than in the control. The effect of forskolin indicates that the drone retina has an adenylate cyclase system that can be activated (see review by Seamon and Daly, 1986). It was therefore important to compare the effects of forskolin and light on glycogen metabolism. This is shown in Fig. 8. Exposure of the retina to 100 μM forskolin for 15 min mimics the effect of light stimulation. As shown in Table I, this activated incorporation of [3H]glycosyls occurred without a significant covalent modification of either phosphorylase or synthetase. In contrast, after 60 min of exposure to forskolin the radioactivity incorporated into glycogen was not different from that in the dark. For this reason, it
is important to note that we did not observe any measurable effect of 100 μM forskolin on: light-induced receptor potential, [K+]o, light-induced Δ[K+]o, intraglial [K+], glial membrane potential, nor QO₂. This suggests that the decrease of labeled glycogen after 60 min of photostimulation or exposure to DNP could be related to a sustained increase of QO₂ in the photoreceptors.

**DISCUSSION**

In this article, we present evidence showing that photostimulation of the isolated drone retina induces an increase of the incorporation and breakdown of [³H]glycosyls into intraglial glycogen, i.e., light induced an increase of glycogen turnover. A similar effect was obtained previously in living drones (Evêquoz et al., 1983). This effect is probably not the consequence of a covalent modification of the two key enzymes of glycogen metabolism, namely synthetase and phosphorylase. Rather, light stimulation seems to exert its effect on the phosphorylation of glucose to glucose-6P and possibly on the rate of cleavage of the disaccharide trehalose to two glucose molecules. In favor of this interpretation is a recent finding showing that repetitive stimulation of the retina caused a 30–40% increase in the formation of [³H]-2DG-6P in the glial cells (Brazitikos and Tsacopoulos, 1990; see also Tsacopoulos et al., 1988) and also that repetitive stimulation of the retina for 15 min (or longer) increased the rate of cleavage of labeled trehalose (see example in Fig. 3 C). Since the phosphorylation of glucose to glucose-6P occurs predominately in the glial cells (see Tsacopoulos et al., 1988) and is an obligatory step for the formation of trehalose (Wyatt, 1967), we hypothesize that trehalose metabolism also occurs in the glia. The nonphosphorylated [³H]glucose and [³H]trehalose diffuse out of the cells in the extracellular space and the bath. Light stimulation by activating the rate of cleavage of [³H]trehalose to [³H]glucose and the phosphorylation of [³H]glucose to [³H]glucose-6P would preserve radiolabeled [³H]glycosyls in the cell that are subsequently incorporated into glycogen. This is the basis of the "push" mechanism proposed on theoretical grounds by El Refai and Bergman (1976) and found experimentally to occur in the starved liver by Van de Werve and Jeanrenaud (1984; see also recent review by Sugden, Holness, and Palmer, 1989).

Photostimulation of slices superfused with metabolic substrate-free Ringer solution quadruples the O₂ consumption (QO₂) for long periods (Tsacopoulos and Poitry, 1982; Tsacopoulos et al., 1987; Fig. 4 of this paper). Quantitative experimental evidence demonstrated that the light-induced ΔQO₂ is tightly coupled to the phototransformation of visual pigment and therefore the light-induced changes of QO₂ are likely to occur in the photoreceptors (Jones and Tsacopoulos, 1987). This was consistent with electron microscopy showing that only the photoreceptors contain significant numbers of mitochondria (also see Introduction). Since photoreceptors contain no detectable amounts of glycogen to maintain this high QO₂ after the isolation of a slice, the glial cells that contain the carbohydrate store of the retina and the capacity to phosphorylate glucose at a much higher rate than photoreceptors, supply more substrate to the mitochondria of the photoreceptors. When photostimulation continues for long periods (up to 60 min) the availability of intracellular [³H]glycosyls is decreased relative to darkness (Tsacopoulos et al., 1988). This should lead to a decrease in the rate of incorporation of [³H]glycosyls into glycogen and thus
to the net degradation of [\(^3\)H]glycogen observed. Of course, this degradation concerns both unlabeled and labeled glycogen (see Devos and Hers, 1979). We have previously reported that after 2 h of repetitive stimulation the concentration of total glycogen (~56 mg/ml of tissue) decreased significantly by 14 ± 4 mg/ml of tissue (mean ± SEM). This amount is sufficient for the carbohydrate requirements calculated from the O\(_2\) consumption (Tsacopoulos et al., 1987). Consequently, in this highly compartmented nervous system, there are necessarily metabolic interactions and travelling of signals between photoreceptor neurons and glial cells.

**K\(^+\) as Signal**

The K\(^+\) hypothesis proposed by Kuffler and Nicholls (1966) seemed applicable first of all because measurements of K\(^+\) with microelectrodes in a variety of nervous tissues showed that neuronal stimulation induces large and rapid increases of [K\(^+\)], (see review by Walz, 1989) which could mobilize nutrients from the glycogen-rich glia to the neurons (Kuwabara and Cogan, 1961; Lajtha, Maker, and Clarke, 1981). The K\(^+\) hypothesis appeared in textbooks and reviews as a paradigm, even though the experimental demonstration was ambiguous or even negative (see recent reviews: Vernadakis, 1988; Walz, 1989). Supporting experimental evidence was reported by Orkand, Bracho, and Orkand (1973) and later by Salem, Hammerschlag, Bracho, and Orkand (1975) showing that raised extracellular [K\(^+\)] affects the oxidative metabolism and [\(^14\)C]glucose uptake by glial cells in the glial preparation obtained from axotomised optic nerves of *Necturus*. It is difficult however to compare these results with ours since in the axon-free preparation glial cells had both glycolysis and mitochondrial respiration. Recently Pentreath and Kai-Kai (1982) reported results from experiments performed on isolated ganglia of snails, showing that electrical stimulation of nerves outside the ganglia or elevation of K\(^+\) induced an increase of uptake and incorporation of 3H-2-DG in glycogen. The authors concluded that the increase of 3H-2-DG metabolism during neuronal stimulation was mediated by K\(^+\). This conclusion is in contrast with the experimental results presented in this paper that have been obtained in another invertebrate nervous system. Raising extracellular K\(^+\) in the dark to a level that causes depolarization of glial cells of the drone retina and increase of intraglial [K\(^+\)] equivalent to that induced by repetitive light stimulation did not mimic the effect of light on either oxidative metabolism of photoreceptors or intraglial glycogen turnover. Increasing extracellular [K\(^+\)] in the snail ganglia probably depolarized the neuronal synapses, thus causing the release of neurotransmitters or other hormones that secondarily affected the neuronal and glial metabolism. Another possibility could be that in the ganglia, a K\(^+\)-induced depolarization causes a large rise of intracellular [Ca\(^{2+}\)], which in turn activates neuronal metabolism (Ritchie, 1973; Hof et al., 1988). In the invertebrate photoreceptors there are also voltage-sensitive Ca\(^{2+}\) conductances (for the glial cells this is not known), but the rise of [Ca\(^{2+}\)], when it occurs, is mainly the consequence of release from intracellular stores (see review by Fain and Lisman, 1981; and Ziegler and Walz, 1989). This provides a possible explanation for the finding that the light-induced rise of QO\(_2\) is much higher than that induced by 50 mM [K\(^+\)] in the bathing solution (see also Fein and Tsacopoulos, 1988).

The cells in which the effect of K\(^+\) on the activities of glycogen enzymes has been
studied in detail are hepatocytes (Hue, Bontemps, and Hers, 1975). Increasing the concentration of K⁺ (replacement of Na⁺ by K⁺) in the incubation medium from 0 to 80 mM primarily inactivates phosphorylase. A further increase of [K⁺] concomitantly activates synthetase. This activation of glycogen synthetase in high [K⁺] is secondary to the inactivation of phosphorylase. In the nervous system of the superfused drone retina, prolonged stimulation failed to induce any change in the activities of phosphorylase or synthetase despite a substantial increase of intraglial [K⁺]. This was intriguing since the basal activities of glycogen enzymes in liver and drone retina measured with the same method in the same batch of samples were similar. In addition, exposure of drone slices to 20 mM of glucose caused, as in liver, a small but significant inhibition of phosphorylase and a concomitant activation of synthetase. It is noticeable, however, that a subtle allosteric activation of glycogen enzymes would be undetectable by the method used here because the exact intracellular environment could not be maintained in vitro.

Our interpretation of the results presented in this paper is that the signal between photoreceptor neurons and glial cells is not K⁺, and whatever this signal is, its targets are not the enzymes of glycogen metabolism, but rather, glycolysis and the rate of glucose phosphorylation.

**cAMP as Signal**

It is well established that the increase of the concentration of cAMP induces phosphorylation and therefore covalent activation of phosphorylase and synthetase (see review by Krebs, 1981). Despite the absence of an effect of light stimulation on the covalent modification of the enzymes of glycogen metabolism in the drone retina, there were still good reasons to consider intracellular cAMP as the key step in metabolic signaling between photoreceptor neurons and glial cells. First, in a nervous preparation without synapses, the squid giant axon, it has been reported that the Schwann glial cells respond electrically to a number of transmitter molecules, including carbachol, octopamine, VIP, and glutamate (Evans, Reale, and Villegas, 1986; Abbott, Hassan, and Lieberman, 1988). Classically these neurotransmitters induce their effects through a rise of cAMP (see review by Rasmussen and Goodman, 1977). Second, it has been shown, at least in yeast cells, that the rise of cAMP, induced for example by exposure to DNP, causes the activation of trehalase (Thevelein, Beullens, Honshoven, Hoebeeck, Detremerie, Griewel, Den Hollander, and Jans, 1987), which is the specific enzyme for the cleavage of trehalose to two glucose molecules. The effects of forskolin and DNP on the cAMP content and glycogen metabolism of the drone retina presented in this article were consistent with the cAMP hypothesis. Inconsistent with the hypothesis was the absence of a measurable rise of cAMP upon photostimulation and the experimental evidence showing that exposure to forskolin and DNP did not induce covalent modification of phosphorylase and synthetase despite a significant rise of intracellular cAMP. One possible explanation of the results could be that trehalase and/or hexokinase can be activated by cAMP, which in turn induces a rise in the availability of [³H]glycosyls to be incorporated in glycogen. However, in physiological conditions, the activation of glucose phosphorylation in the glial cells should be a cAMP independent process. In this context it is of interest that recently Pearce, Morrow, and Murphy (1988)
presented evidence showing that in cultured astrocytes the incorporation of \textsuperscript{3}H-2DG in glycogen is affected by two metabolic systems: the activation of protein kinase C by phorbol ester and the activation of adenylate cyclase by forskolin.

Presently, we favor the exploration of the hypothesis that after light stimulation, a change in the gradient of the substrate between the mitochondrial surface in the photoreceptors and glial cytoplasm is a sufficient signal for the observed stimulation of intraglial glucose metabolism.

\textit{Comparison with Mammalian Brain}

When rodent cortical slices are incubated in a medium carrying \textsuperscript{3}H\textsuperscript{2}glucose, \textsuperscript{3}H\textsuperscript{2}glycosyls are incorporated into glycogen (Quach, Rose, and Schwartz, 1978; Quach, Duchemin, Rose, and Schwartz, 1980; Quach, Rose, Duchemin, and Schwartz, 1982; Magistretti, Morrison, Shoemaker, Sapin, and Bloom, 1981). Addition of a potent neurotransmitter (histamine, serotonin, VIP) in the incubation medium induced (in the presence of \textsuperscript{3}H\textsuperscript{2}glucose) a rapid fall of \textsuperscript{3}H\textsuperscript{2}glycogen. This is in contrast with the observations made in the superfused drone retina and the retina of living drone (Evêquoz et al., 1983). In intact rodents, Watanabe and Passonneau (1973) showed that administration of hydrocortisone increases \textsuperscript{3}H\textsuperscript{2}glycogen turnover without any effect on the interconversion (synonymous to activation) of phosphorylase \textit{a} and \textit{b} and synthetase I and D. They concluded that the rate of glycogen synthesis and degradation in the brain is possibly controlled by the fine adjustments of concentrations of metabolites and cofactors. Goldberg and O'Toole (1969) have proposed a similar mechanism. From this point of view, control of glycogen metabolism in the drone retina is similar to that in the brain. The fundamental difference between drone retina and mammalian brain is that the glia of the former contain large amounts of two distinct carbohydrate pools, trehalose and glycogen, that draw glycosyls from the same source, the glucose-6-P. When \textsuperscript{3}H\textsuperscript{2}glucose enters glial cells it is transformed to \textsuperscript{3}H\textsuperscript{2}glucose-6P which in large part is used to make \textsuperscript{3}H\textsuperscript{2}trehalose, with only a small fraction being incorporated into glycogen. When the photoreceptor neurons are stimulated they send a signal to the glial cells that stimulates the phosphorylation of glucose. Thus, glial metabolism meets the carbohydrate requirement of the \textsubscript{O}2 consumption of photoreceptor neurons. The existence of two distinct intracellular pools of carbohydrate provides a powerful security system for the maintenance of the oxidative metabolism of the photoreceptors, important for the functional integrity of these cells (Baumann and Mauro, 1973; Dimitracos and Tsacopoulos, 1985).

We thank Mr. Ph. Perrottet for expert technical assistance in the biochemical experiments, Dr. F. Assimacopoulos-Jeannet for advice and comments, Dr. J. A. Coles and colleagues in our laboratory for critical comments on the manuscript, and especially Mr. S. Poitry for making computer programs and calculations.

This research was supported by the Swiss National Science Foundation grant 3.050-0.87, and the Georges Kernen Foundation.

\textit{Original version received 5 March 1990 and accepted version received 22 February 1991.}
EVEQUOZ-MERCIER AND TSACOPOULOS  Metabolism in Glial Cells of Honeybee Retina 513

REFERENCES

Abbott, N. J., S. Hassan, and E. M. Lieberman. 1988. Evidence for glutamate as the mediator of axon-Schwann cell interactions in the isolated giant axon of the squid. Journal of Physiology. 398:63P. (Abstr.)

Baumann, F. 1974. Electrophysiological properties of the honeybee retina. In The Compound Eye and Vision of Insects. G. A. Horridge, editor. Clarendon Press, Oxford. 53–74.

Baumann, F., and A. Mauro. 1973. Effects of hypoxia on the change in membrane conductance evoked by illumination in arthropod photoreceptors. Nature New Biology. 244:146–148.

Baumann, O., and B. Walz. 1989. Topography of Ca2+-sequestering endoplasmic reticulum in the photoreceptors and pigmented glial cells in the compound eye of the honeybee drone. Cell and Tissue Research. 255:511–522.

Brazitikos, P. D., and M. Tsacopoulos. 1990. Light stimulation of the photoreceptors activates phosphorylation of H-2-deoxyglucose (H-2DG) in the glial cells of the drone retina. Investigative Ophthalmology and Visual Science. 31:80. (Abstr.)

Coles, J. A. 1989. Function of glial cells in the retina of the honeybee drone. Glia. 2:1–9.

Coles, J. A., and R. K. Orkand. 1983. Modification of potassium movement through the retina of the drone (Apis mellifera) by glial uptake. Journal of Physiology. 340:157–174.

Coles, J. A., R. K. Orkand, C. L. Yamate, and M. Tsacopoulos. 1986. Free concentrations of Na, K and Cl in the retina of the honeybee drone: stimulus-induced redistribution and homeostasis. Annals of the New York Academy of Sciences. 481:303–317.

Coles, J. A., and G. Schneider-Picard. 1989. Increase in glial intracellular K+ in drone retina caused by photostimulation but not mediated by an increase in extracellular K+. Gla. 2:213–222.

Coles, J. A., and M. Tsacopoulos. 1979. K+ activity in photoreceptors, glial cells and extracellular space in the drone retina: changes during photostimulation. Journal of Physiology. 290:525–549.

Coles, J. A., and M. Tsacopoulos. 1981. Ionic and possible metabolic interactions between sensory neurones and glial cells in the retina of the honeybee drone. Journal of Experimental Biology. 95:75–92.

Devos, P., and H. G. Hers. 1979. A molecular order in the synthesis and degradation of glycogen in the liver. European Journal of Biochemistry. 99:161–167.

De Wulf, H., and H. G. Hers. 1968. The interconversion of liver glycogen synthase a and b in vitro. European Journal of Biochemistry. 6:552–557.

Dimitracos, S. A., and M. Tsacopoulos. 1985. The recovery from a transient inhibition of the oxidative metabolism of the photoreceptors of the drone (Apis mellifera). Journal of Experimental Biology. 119:165–181.

El-Refai, M., and R. N. Bergman. 1976. Simulation study of control of hepatic glycogen synthesis by glucose and insulin. American Journal of Physiology. 231:1608–1619.

Evans, P. D., Y. Reale, and J. Villegas. 1986. Peptidergic modulation of the membrane potential of the Schwann cell of the squid giant nerve fibre. Journal of Physiology. 379:61–82.

Evéquoz, V., A. Stadelmann, and M. Tsacopoulos. 1983. The effect of light on glycogen turnover in the retina of the intact honeybee drone (Apis mellifera). Journal of Comparative Physiology. 150:69–75.

Fain, G. L., and J. E. Lisman. 1981. Membrane conductances of photoreceptors. Progress in Biophysics and Molecular Biology. 37:91–147.

Fein, A., and M. Tsacopoulos. 1988. Activation of neuronal oxidative metabolism by calcium ions. Nature. 331:437–440.

Goldberg, N. D., and A. C. O'Toole. 1969. The properties of glycogen synthetase and regulation of glycogen biosynthesis in rat brain. Journal of Biological Chemistry. 244:3053–3061.
Hof, P. R., E. Pascale, and P. J. Magistretti. 1988. K⁺ at concentrations reached in the extracellular space during neuronal activity promotes a Ca²⁺-dependent glycogen hydrolysis in mouse cerebral cortex. *Journal of Neuroscience.* 8:1922–1928.

Hue, L., F. Bontemps, and H. G. Hers. 1975. The effect of glucose and of potassium ions on the interconversion of the two forms of glycogen phosphorylase and of glycogen synthase in isolated rat liver preparation. *Biochemical Journal.* 152:105–114.

Jones, G. J., and M. Tsacopoulos. 1987. The response to monochromatic light flashes of the oxygen consumption of honeybee drone photoreceptors. *Journal of General Physiology.* 89:791–813.

Krebs, E. G. 1981. Phosphorylation and dephosphorylation of glycogen phosphorylase: a prototype for reversible covalent enzyme modification. *Current Topics in Cellular Regulation.* 18:401–419.

Kuffler, S. W., and J. G. Nicholls. 1966. The physiology of neuroglial cells. *Ergebnisse der Physiologie.* 57:1–90.

Kuffler, S. W., and J. G. Nicholls. 1976. From Neuron to Brain. Sinauer Associates, Inc., Sunderland, MA.

Kuffler, S. W., J. G. Nicholls, and R. K. Orkand. 1966. Physiological properties of glial cells in the central nervous system of amphiabia. *Journal of Neurophysiology.* 29:768–787.

Kuwabara, I., and D. Cogan. 1961. Retinal glycogen. *Archives of Ophthalmology.* 66:680–688.

Lajtha, A. L., H. S. Maker, and D. O. Clarke. 1981. Metabolism and transport of carbohydrates and amino acids. In *Basic Neurochemistry.* 3rd Ed. G. Siegel et al., editors. Little, Brown and Co., Boston. 329–353.

Magistretti, P. J., H. H. Morrison, W. J. Shoemaker, V. Sapin, and F. E. Bloom. 1981. Vasoactive intestinal polypeptide induces glycogenolysis in mouse cerebral cortical slices: a possible regulatory mechanism for the local control of energy metabolism. *Proceedings of the National Academy of Sciences.* 78:6535–6539.

Muri, R. B., and G. J. Jones. 1983. Microspectrophotometry of single rhabdoms in the retina of the honeybee drone (*Apis mellifera d*). *Journal of General Physiology.* 82:469–496.

Orkand, P. M., H. Bracho, and R. K. Orkand. 1973. Gliarial metabolism: alteration by potassium levels comparable to those during neural activity. *Brain Research.* 55:467–471.

Pearce, B., C. Morrow, and S. Murphy. 1988. A role for protein kinase C in astrocyte glycogen metabolism. *Neuroscience Letters.* 9:191–196.

Pentreath, V. W., and M. A. Kai-Kai. 1982. Significance of the potassium signal from neurones to glial cells. *Nature.* 295:59–61.

Perrelet, A. 1970. The fine structure of the retina of the honeybee drone. *Zeitschrift für Zellforschung und Mikroskopische Anatomie.* 108:530–562.

Quach, T. T., A. M. Duchemin, C. Rose, and J. C. Schwartz. 1980. [³H]Glycogen hydrolysis elicited by histamine in mouse brain slices: selective involvement of H₄ receptors. *Molecular Pharmacology.* 17:301–308.

Quach, T. T., C. Rose, A. M. Duchemin, and J. C. Schwartz. 1982. Glycogenolysis induced by serotonin in brain: identification of a new class of receptor. *Nature.* 298:373–375.

Quach, T. T., C. Rose, and J. C. Schwartz. 1978. [³H]Glycogen hydrolysis in brain slices: responses to neurotransmitters and modulation of noradrenaline receptors. *Journal of Neurochemistry.* 30:1335–1341.

Rasmussen, H., and D. B. P. Goodman. 1977. Relationships between calcium and cyclic nucleotides in cell activation. *Physiological Reviews.* 57:421–509.

Ritchie, J. M. 1973. Energetic aspects of nerve conduction: the relationships between heat production, electrical activity and metabolism. *Progress in Biophysics and Molecular Biology.* 26:149–187.

Salem, R. D., R. Hammerschlag, H. Bracho, and R. K. Orkand. 1975. Influence of potassium ions on accumulation and metabolism of [¹⁴C]glucose by glial cells. *Brain Research.* 86:499–503.
Seamon, K. B., and J. W. Daly. 1986. Forskolin: its biological and chemical properties. *Advances in Cyclic Nucleotide Protein Phosphorylation Research.* 20:1–150.

Shimizu, H., J. W. Daly, and C. R. Creveling. 1969. A radioisotopic method for measuring the formation of adenosine 3',5'-cyclic monophosphate in incubated slices of brain. *Journal of Neurochemistry.* 16:1609–1619.

Sugden, M. C., M. J. Holness, and T. N. Palmer. 1989. Fuel selection and carbon flux during the starved-to-fed transition. *Biochemical Journal.* 263:313–323.

Thevelein, J. M., M. BeuUens, F. Honshoven, G. Hoebeeck, K. Detremerie, B. Griewel, J. A. Den Hollander, and A. W. H. Jans. 1987. Regulation of the cAMP level in the yeast Saccharomyces cerevisiae: intracellular pH and the effect of membrane depolarizing compounds. *Journal of General Microbiology.* 133:2197–2205.

Thomas, J. A., K. K. Schlender, and J. Larner. 1968. A rapid filter paper assay for UDP glucose-glycogen glucosyltransferase, including an improved biosynthesis of UDP-14C-glucose. *Analytical Biochemistry.* 25:486–499.

Tsacopoulos, M., J. A. Coles, and G. Van de Werve. 1987. The supply of metabolic substrate from glia to photoreceptors in the retina of the honeybee drone. *Journal of Physiology (Paris).* 82:279–287.

Tsacopoulos, M., V. Evéquoz-Mercier, P. Perrottet, and E. Buchner. 1988. Honeybee retinal glial cells transform glucose and supply the neurons with metabolic substrate. *Proceedings of the National Academy of Sciences.* 85:8727–8731.

Tsacopoulos, M., and S. Poitry. 1982. Kinetics of oxygen consumption after a single flash of light in photoreceptors of the drone (Apis mellifera). *Journal of General Physiology.* 80:19–55.

Tsacopoulos, M., S. Poitry, and A. Borsellino. 1981. Diffusion and consumption of oxygen in the superfused retina of the drone (Apis mellifera) in darkness. *Journal of General Physiology.* 77:601–628.

Van de Werve, G., and B. Jeanrenaud. 1984. Synthase activation is not a prerequisite for glycogen synthesis in the starved liver. *American Journal of Physiology.* 247:E271–E275.

Van de Werve, G., G. Van den Berghe, and H. G. Hers. 1974. A simplified procedure for the assay of adenosine 3',5'-monophosphate by the activation of liver phosphorylase. *European Journal of Biochemistry.* 41:97–102.

Vernadakis, A. 1988. Neuron-glia interrelations. *International Review of Neurobiology.* 30:149–224.

Walz, W. 1989. Role of glial cells in the regulation of the brain ion microenvironment. *Progress in Neurobiology.* 33:309–353.

Watanabe, H., and J. V. Passonneau. 1973. Factors affecting the turnover of cerebral glycogen and limit dextrin in vivo. *Journal of Neurochemistry.* 20:1543–1554.

Wyatt, G. R. 1967. The biochemistry of sugars and polysaccharides in insects. *Advances in Insect Physiology.* 4:287–360.

Ziegler, A., and B. Walz. 1989. Analysis of extracellular calcium and volume changes in the compound eye of the honeybee drone, Apis mellifera. *Journal of Comparative Physiology A.* 165:697–709.