Ammonium and Glutamate Released by Neurons Are Signals Regulating the Nutritive Function of a Glial Cell

Marcos Tsacopoulos, Carol L. Poitry-Yamate, and Serge Poitry

1Experimental Ophthalmology Laboratory and Departments of 2Physiology and 3Pharmacology, University of Geneva Medical School, 1211 Geneva 4, Switzerland

Gliol cells transform glucose to a fuel substrate taken up and used by neurons. In the honeybee retina, photoreceptor neurons consume both alanine supplied by glial cells and exogenous proline. Ammonium (NH$_4^+$) and glutamate, produced and released in a stimulus-dependent manner by photoreceptor neurons, contribute to the biosynthesis of alanine in glia. Here we report that NH$_4^+$ and glutamate are transported into glia and that a transient rise in the intraglial concentration of NH$_4^+$ or of glutamate causes a net increase in the level of reduced nicotinamide adenine dinucleotides [NAD(P)H]. Biochemical measurements indicate that this is attributable to activation of glycolysis in glial cells by the direct action of NH$_4^+$ and glutamate on at least two enzymatic reactions: those catalyzed by phosphofructokinase (PFK; ATP:fructose-6-phosphotransferase, EC 2.7.1.11) and glutamate dehydrogenase (GDH; L-glutamate:NAD oxidoreductase, deaminating; EC 1.4.1.3). This activation leads to an increase in the production and release of alanine by glia. This signaling, which depends on the rate of conversion of NH$_4^+$ and glutamate to alanine and $\alpha$-ketoglutarate, respectively, in the glial cells, raises the novel possibility of a tight regulation of the nutritive function of glia.

Key words: NADH; pH; fluorescence imaging; NH$_4^+$ transport; glutamate transport; retina

Growing evidence suggests that astrocytes, by their physiological interactions with neurons, are involved in mammalian brain function and energy metabolism (Pellerin and Magistretti, 1994). However, the complex structural interactions among astrocytes, neurons, and capillaries make functional quantitation difficult at the cellular level. Although cultured astrocytes have provided a basis for understanding brain energy metabolism, there is presently no clear reported case in which a molecular signal physiologically released from neurons actually exerts a fine control of glial metabolism. Glutamate, a major excitatory neurotransmitter in the CNS, is a case in point. Extensive electrophysiological work on acutely isolated vertebrate glia has demonstrated that glutamate is taken up electrogenically by glia (Brew and Attwell, 1987). In cultured astrocytes, this uptake activates glucose uptake and metabolism, and it has been suggested that this activation is attributable to increased activity of a glial-specific Na$^+$/K$^+$ ATPase (Pellerin and Magistretti, 1994). However, alternative possibilities can be evoked. First, because the enzyme converting glutamate to glutamine at the expense of ammonia and ATP apparently is localized exclusively in glia, it is expected that an increased glutamate uptake into astrocytes would lead to a decrease in intracellular levels of ATP and ammonia. The question remains whether this decrease constitutes a primary metabolic signal and whether these levels are restored in astrocytes by increasing oxidative metabolism and ammonia uptake. Second, glutamate uptake causes the pH in glial Müller cells to go acid, and it was suggested that this transient pH change may be a signal to alter glial metabolism (Bouvier et al., 1992). Thus, although glutamate affects the metabolic state of glia, whether it further constitutes a signal coupling neuron function and metabolism to those in glia only can be surmised. We approached the problem of metabolic signaling between neurons and glia in honeybee retina because direct analysis performed in this preparation already has proven to provide an unsurpassed basis for parallel studies in mammals (Poitry-Yamate et al., 1995).

The honeybee retina has been an appropriate model system for exploring metabolic interactions between neurons and glial cells, because light-sensitive photoreceptor neurons have almost all of the mitochondria, whereas glial cells are packed with glycogen. Moreover, although glial cells do not respond directly to light (Tsacopoulos et al., 1987; Coles, 1989), their glycogen metabolism is modified when photoreceptors are photo-stimulated (Évèquoz-Mercier and Tsacopoulos, 1991). Furthermore, glia transform glucose to alanine, which, in turn, is taken up and used by photoreceptors (Tsacopoulos et al., 1994). By analogy in mammals, evidence indicates that astrocytes fuel neurons with lactate (Larrabee, 1983; Poitry-Yamate et al., 1995; Tsacopoulos and Magistretti, 1996). In honeybee retina, photoreceptors also consume proline (Tsacopoulos et al., 1994), and this consumption leads to the production and release, in a stimulus-dependent manner, of NH$_4^+$ and glutamate, which, in turn, contribute to the biosynthesis of alanine in glia (Tsacopoulos et al., 1997). Here we show that ammonium (NH$_4^+$) and glutamate released by photoreceptors return to glial cells not simply for nitrogen conservation but as information signals.

MATERIALS AND METHODS

Materials. [U-$^{14}$C]Glutamate was purchased from Amersham (Zürich, Switzerland; specific activity 266 mCi:mmol, 1 Ci = 37 GBq). [U-$^{14}$C]Glucose was purchased from DuPont/NEN (Boston, MA; specific activity 298 mCi:mmol). 2,7-Biscarboxyethyl-5(6)-carboxyfluorescein-AM
(BCECF-AM) was obtained from Molecular Probes (Leiden, The Netherlands). Other chemicals or enzymes have been used previously (Tsacopoulos et al., 1994; Veuthey et al., 1994) and were obtained from Sigma (Buchs, Switzerland), Fluka (Buchs, Switzerland), or Calbiochem (Basel, Switzerland).

Preparation of retinal homogenates and a mitochondrial fraction from drone retinal slices. For the measurement of ammonia, retinal slices, retinal homogenates, and the mitochondrial fraction were prepared as previously described in Tsacopoulos et al. (1987) and Veuthey et al. (1994). Briefly, nonretinal portions of a slice were removed by micromanipulation using fine forceps, leaving a pure retinal slice. Batches of retinal slices then were homogenized manually. Parts of these homogenates were processed further by subcellular fractionation (see Veuthey et al., 1994) for isolation of the mitochondrial fraction. NH$_4^+$ was assayed with dansyl-Cl (5-dimethylamino-1-naphthalene-sulfonyl chloride) derivatization and fluorescence detection (HP 1090 HPLC system, Hewlett-Packard, Palo Alto, CA) to precolumn derivatization with orthophthaldehyde and fluorescence detection (HP 1090 HPLC system, Hewlett-Packard, Palo Alto, CA) (Tsacopoulos et al., 1994; Poitry-Yamart et al., 1995).

RESULTS

Ammonia transport and signaling

Ammonia essentially is produced in the mitochondria of photoreceptors (Fig. 1, left) and subsequently is released into the extracellular space (Tsacopoulos and Poitry, 1995; Coles et al., 1996a; Tsacopoulos et al., 1997). Previously, we showed that light stimulation of honeybee retinal slices or exposure to dinitrophephrine (both induce an increase in the O$_2$ consumption of photoreceptors; Tsacopoulos et al., 1987) causes an increased release of NH$_4^+$ into the extracellular space. Recent measurements with NH$_4^+$- and pH-sensitive microelectrodes showed that photoreceptors, within seconds of an increase in their oxidative metabolism, release an increased amount of NH$_4^+$/NH$_3$, which, in turn, induces an increase in pH in the interstitial cleft and NH$_4^+$/NH$_3$ entry into presumably glial cells (Coles et al., 1996a). In keeping with these results, we observed that the intraglial pool of NH$_4^+$ varied with the level of NH$_4^+$ in the extracellular space (Fig. 1a, right). In addition, when glial cells 3–8 hr after isolation were puffed with NH$_4$Cl, pH$_i$ became acid (Fig. 1e), indicating that either NH$_4^+$, rather than NH$_3$, entered the cells (Kikeri et al., 1989; Coles et al., 1996a) or that glycolysis was activated. This acidification was smaller than that measured under similar conditions after a longer (2 min) exposure of the cells to 2 mM NH$_4^+$ (Coles et al., 1996b). As shown in Figure 1h,c, puffs of NH$_4$Cl at the glial surface caused up to a 100% increase in the fluorescence of reduced nicotinamide adenine dinucleotides [i.e., NADH and/or NADPH, abbreviated in this text as NAD(P)H]. Exposure to 8 mM BaCl$_2$ from the bath reversely suppressed this effect (Fig. 1c). Suppression also was observed on cell exposure to Na$^+$-free (Fig. 2b) or Cl$^-$-free solution (Fig. 1d), consistent with a strong decrease of the NH$_4^+$-induced transient acidosis (Fig. 1e). Thus, NH$_4^+$ entry into these glial cells shares properties with that in kidney cells (Kinne et al., 1986; Kikeri et al., 1989; Amlal et al., 1994), suggesting that it also may be mediated by a Na$^+$/K$^+$/2 Cl$^-$/H$^+$ exchange system.
cotransporter and a barium-sensitive K\(^+\)/H\(^+\) exchanger. This uptake system allows the entry of NH\(_4^+\) into glia, but not its exit. Thus, the slow recovery of [NH\(_4^+\)] after a transient accumulation (Fig. 1a) probably reflects its chemical fixation in the process of alanine biosynthesis (see below). The extreme scarcity of mitochondria in drone glia (Tsacopoulos et al., 1987; Coles, 1989), as well as the relative homogeneity of the basal fluorescence along the cells (Fig. 1b, right), favors the idea that the measured fluorescence was cytosolic. It is, however, difficult to determine whether it was attributable to NADH or to NADPH, because the spectral properties of these compounds are almost identical. We suggest that the rapid rise of fluorescence in response to puffing NH\(_4^+\)Cl reflected an increase in the NADH level consecutive to an activation of glycolysis by NH\(_4^+\) ions. We do not exclude that NADH in excess then was converted to NADPH, but this would require the presence of a transhydrogenase, which was not determined here. When the kinetics of intraglial ΔNAD(P)H and ΔpH in response to puffs of NH\(_4^+\)Cl were compared, no clear differences were revealed in their rising phases. However, citrate caused a larger and longer-lasting acidification than NH\(_4^+\) but a correspondingly smaller change in NAD(P)H fluorescence (Fig. 1d,e). Hence, we suggest that transient increases in NAD(P)H levels are elicited by a direct, pH-independent action of NH\(_4^+\) on a glycolytic enzyme. Phosphofructokinase (PFK; ATP:D-fructose-6-phosphotransferase, EC 2.7.1.11) is a possible target because it has been shown in mammalian brain extracts that NH\(_4^+\) acts on it as an allosteric activator (Lowry and Passonneau, 1966). In agreement, activation of glycolysis in rat brains was reported when NH\(_4^+\) concentration in the blood was raised from 0.01 to 1.74 mM (Hawkins et al., 1973).

Figure 1. NH\(_4^+\) produced by oxidative metabolism in photoreceptors is taken up by glial cells, where it decreases pH\(_i\) and increases NAD(P)H. a, Left panel, [NH\(_4^+\)] in whole retinal slices and in subcellular compartments. A, Fresh slice, immediately frozen; B, retinal homogenate; C, mitochondrial fraction of retinal homogenate. Right panel, Dependence of [NH\(_4^+\)] in freshly isolated glial cells on incubating conditions. D, After 1 hr in 0-NH\(_4^+\), Ringer’s solution; E, after 1 hr in 400 μM NH\(_4^+\); F, 1/2 hr after returning to 0-NH\(_4^+\); G, at 18 hr after returning to 0-NH\(_4^+\). Values are mean ± SEM (n = 10). b, Left panel, Increase of NAD(P)H fluorescence in glial cells in response to puffing NH\(_4^+\)Cl; the increase was larger and faster near the puff tip (traces 2 and 3). Puff duration, 20 sec. Arrows indicate times of capture of fluorescence images shown on right panel. Numbers 1–3 correspond to analyzed cell regions (right panel, top). Right panel, Bundle of five isolated glial cells (top) and image of their basal (middle) and peak (bottom) NAD(P)H fluorescence captured after NH\(_4^+\)Cl puff. Pseudocolor scale of NAD(P)H fluorescence levels, with increases toward red. Asterisk overlies NH\(_4^+\)Cl puffer. c, Exposure of glial cells to BaCl\(_2\) (8 mM) reversibly suppressed NH\(_4^+\)Cl-induced ΔNAD(P)H response. d, NH\(_4^+\) at indicated concentrations, but not citrate, lead to significant NAD(P)H increases. Cl\(^-\)-free Ringer’s solution suppresses the effect of NH\(_4^+\). e, pH\(_i\) changes elicited by same substances as in d. Results in d and e are means of n (superimposed on bars) measurements ± SEM.
Glutamate transport and signaling

There is strong evidence that glutamate production and its non-vesicular release in the synapse-free honeybee retina (Coles, 1989; Tsacopoulos et al., 1994) are predominantly neuronal. First, analysis of the retinal interstitial fluid in living honeybees showed that the concentration of glutamate there is 10 times higher than in the hemolymph (Cardinaud et al., 1994), indicating retinal production and release. Second, when the intact retina is exposed to [15N]proline, which is consumed in the mitochondria of photoreceptors (Tsacopoulos et al., 1994), 15N-glutamate is produced and rapidly

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**Figure 2.** Glutamate induces in glial cells an increase of NAD(P)H fluorescence and a decrease of pH. a, A 20 sec puff of 0.5 mM glutamate (Glu), but not of 0.5 mM aspartate (Asp), induces NAD(P)H fluorescence increase. Puffing glutamate after aspartate (recovery) led to a slower rise in NAD(P)H fluorescence. b, Effect of glutamate (1 mM) on NAD(P)H fluorescence was reversibly suppressed when Na⁺ in the bath was replaced by equimolar amounts of choline. During choline exposure, puffing NH₄Cl in choline–Ringer’s solution (second time bar) had no effect on fluorescence. Response to glutamate partially recovered, 5 min after return to normal Na⁺ (recovery). Lower panel, Bundle of four glial cells and tips of two puffers. c, Puffing 1 mM NH₄Cl after 1 mM glutamate induces a further increase in NAD(P)H fluorescence. Puff duration in a–c was 20 sec. d, Glutamate at three concentrations, but not aspartate, leads to significant NAD(P)H increases. e, Both glutamate and aspartate induce a significant, transient, intracellular acidosis in glial cells. Results in d and e are means of n (superimposed on bars) measurements ± SEM. f, Transport of radiolabeled [U-14C]glutamate into glia is Na⁺-dependent. Cells were exposed to 50 μM [U-14C]glutamate for the times indicated, in either Na⁺ or choline containing Ringer’s solution. Each time point represents four separate experiments (4 × 4 retinas) ± SEM.
released into the extracellular space (Tsacopoulos et al., 1997). Third, freshly isolated glia do not produce and release extracellularly measurable amounts of glutamate (Fig. 3a, right), and [glu] in isolated glial cells is ∼20-fold lower (Fig. 3a, left) than that measured in retinal slices (Tsacopoulos et al., 1994). Fourth, using an enzyme-based glutamate-sensitive microelectrode (Innocent, 1995) positioned near the surface of freshly isolated honeybee photoreceptors (Tsacopoulos et al., 1994), we measured a glutamate concentration of 2–4 μM (3 cells), whereas it was virtually zero in the surrounding bath. These findings suggest that glial cells take up glutamate released by photoreceptors. Because the production of alanine from glucose is coupled to the recycling of NAD(P)^+ via the activity of glutamate dehydrogenase (GDH; L-glutamate:NAD oxidoreductase, deaminating; EC 1.4.1.3) in glial cells (Tsacopoulos et al., 1994; Veuthey et al., 1994) and because NH_4^+/NH_3 feeds into this reaction consuming NAD(P)H (Fahien et al., 1971), we tested whether glutamate enters glial cells and modulates NAD(P)H. Puffing glutamate (0.25–1 mM) onto individual glial cells caused a net increase in NAD(P)H fluorescence (Fig. 2a–d), as would be expected if glycolysis were activated and GDH activity were decreased via product inhibition. Consistent with this hypothesis, when NH_4^+ was puffed 20 s after glutamate, NAD(P)H fluorescence increased further (Fig. 2c). The effect of glutamate on NAD(P)H was reversibly suppressed when Na^+ in the bathing solution was replaced by choline (Fig. 2b), indicating that glutamate enters glial cells through a high-affinity Na^+-dependent glutamate transport system similar to that already described for vertebrate glial cells (Brew and Attwell, 1987). Furthermore, cell exposure for ∼40 min to 1 mM β-methyl-aspartate, an inhibitor of glutamate transporter (Kaani and Hediger, 1992), irreversibly suppressed the glutamate-induced ΔNAD(P)H without significantly changing basal NAD(P)H fluorescence. To assess whether glutamate is deaminated oxidatively to α-ketoglutarate as a consequence of product inhibition and Na^+-dependent entry, we incubated bundles of glial cells with radiolabeled glutamate and followed its uptake and transformation over time (Fig. 2f). There was a rapid uptake of [U-14C]glutamate, and within 20 min the amount of [U-14C]glutamate that had accumulated in the cells approached a plateau (Fig. 2f), in part because there was in parallel a production of 14C-α-ketoglutarate (0.68 ± 0.17 mmol/l of glia/min; n = 16 cell batches), which was released mainly in the bath. (Remarkably, there was no 14C-glutamine synthesized in these experiments, indicating that glutamine synthetase is not active in these glial cells.) This is consistent with a sustained uptake and metabolism of glutamate by glia. The accumulation of [U-14C]glutamate was strongly Na^+-dependent in accordance with the Na^+-dependence of the NAD(P)H response (Fig. 2b,f).

The metabolic effect of glutamate in glia is not directly pH-dependent

In vertebrate retinal glial cells, exposure to glutamate causes a transient acidosis, probably because the glutamate uptake carrier countertransport pH-changing anions (OH^- or HCO_3^-) (Bouvier et al., 1992). Because both mitochondria and carbonic anhydrase are absent in honeybee glial cells (Tsacopoulos et al., 1987; Walz, 1988), this countertransport is expected to be limited to OH^- when these cells are isolated. We show that the glutamate-induced acidosis was mild (Fig. 2e), in comparison with that recorded in vertebrate cells (Bouvier et al., 1992). Aspartate, which uses the same transport carrier as glutamate (Christensen, 1990; Bouvier et al., 1992), caused a mild, transient acidosis of similar amplitude to that induced by glutamate (Fig. 2e) but smaller than that caused by the weak acid citrate (Fig. 1e). In sharp contrast to glutamate, however, aspartate (Fig. 2a,d) and citrate (Fig. 1d) did not induce significant NAD(P)H fluorescence increases. It seems, therefore, that the glutamate-induced increase in NAD(P)H is attributable to a direct effect on a specific intracellular enzymatic target, probably cytosolic GDH (Veuthey et al., 1994), and not through intracellular pH changes as suggested before (Bouvier et al., 1992).
Biochemical evidence of the metabolic effects of NH$_4^+$ and glutamate in glia

In honeybee glial cells, where pyruvate is not reduced to lactate (Tsacopoulos et al., 1987), GDH works in tandem with alanine aminotransferase (ALAT; l-alanine-2-oxoglutarate aminotransferase; EC 2.6.1.2) to produce alanine and NAD(P)$^+$ (Fahien et al., 1971; Tsacopoulos et al., 1994; Veuthey et al., 1994; Tsacopoulos and Magistretti, 1996). The concentration of pyruvate in these cells is low (below 0.2 mM). Consequently, the rate of glycolytic production of pyruvate and the supply from the extracellular space of glutamate at noninhibitory levels for GDH (Fahien et al., 1971). We provide biochemical support to this hypothesis: exposing glial cells to a mixture of 1 mM NH$_4^+$ and 1 mM glutamate induced a large increase in the biosynthesis of alanine (Fig. 3a). Most significantly, the amount of alanine released into the bath under these conditions increased fivefold as compared with controls, i.e., with no glutamate and no NH$_4^+$ added to the bath (Fig. 3a, right). The effect of NH$_4^+$ alone was significant but was less important than when combined with glutamate (Fig. 3a). We further asked whether alanine is also produced from glycogen stores. Radiolabeled [U-14C]glucose therefore was added to the glial bath and thereby competed with glycogen pools as the substrate for alanine formation (Fig. 3b). The specific activity calculated for intracellular alanine was very small at two different concentrations of [U-14C]glucose, consistent with the idea that the substrate for its formation is mostly glycogen. The specific activity of alanine in the bath, however, was >10-fold higher than outside the cells (Fig. 3b, bottom panels), indicating that alanine is produced from both exogenous [U-14C]glucose and glycogen but that alanine produced from exogenous glucose is released more rapidly into the bath (Poitry-Yamate et al., 1995). The addition of 1 mM NH$_4^+$ and 1 mM glutamate caused as much as a fivefold decrease in the specific activity of alanine in the bath, whereas inside the cells the increase was further (Fig. 3b, bottom panels). This is probably the consequence of a massive hydrolysis of glycogen caused by the pulling effect of increased glycolysis (Évéquoz-Mercier and Tsacopoulos, 1991). This mechanism is similar to that operating in mammalian astrocytes, both fresh and cultured, with respect to glutamate signaling and lactate formation and release (Parpura et al., 1994; Pellerin and Magistretti, 1994; Poitry-Yamate et al., 1995; Tsacopoulos and Magistretti, 1996).

**DISCUSSION**

We have explored the signal(s) by which neurons communicate to glia their need for energy substrate and at what level the signals exert their metabolic effect in glia. The experimental evidence presented in this paper shows that ammonium and glutamate released physiologically by neurons inform glial cells to increase their production and release of energy substrate.

Baseline levels of ammonia in honeybee retinal slices or homogenates are higher than in mammalian brain (Cooper and Plum, 1987). However, as we have shown, the major part of ammonia is found in the mitochondria, and this may be related to the consumption of proline in this tissue. Although it is difficult to estimate the cytosolic and extracellular levels of ammonia in the intact retina, it is quite likely that they are much less than the values reported in Figure 1a.

The model diagram based on our findings (Fig. 4) describes a novel way by which this arises. Ammonium and glutamate exert their signaling effect intracellularly and synchronously on three enzymatic reactions in the glia: during activation, NH$_4^+$ and glutamate operating at two sites (PFK and ALAT) accelerate the production of alanine from pyruvate and of α-ketoglutarate from glutamate. Alanine and α-ketoglutarate are taken up by neurons and contribute there to the homeostasis of the mitochondrial redox potential (Tsacopoulos et al., 1994). At the same time NH$_4^+$ and glutamate have antagonistic effects at a third site (GDH). Thus, the overall effect of NH$_4^+$ on glial metabolism is to increase the production of alanine, and that of glutamate is to adjust this production to the metabolic needs of photoreceptors. In intact tissue, the operation of this fine control during neuronal activity probably would result in smaller and faster ΔNAD(P)H than those recorded in isolated cells, and the flux through GDH in glia might be expected to rise and fall with shifts of internal conditions.
concentrations of glutamate and/or NH₄⁺. This signaling depends on functional plasma membrane transport systems for NH₄⁺ and glutamate in glial cells and leads to the transformation of these molecules intracellularly. In metabolically active glial cells containing enough glycogen or in the presence of extracellular glucose, NH₄⁺ and glutamate taken up from the extracellular space do not accumulate intracellularly in excess, because they are transformed continuously to alanine and α-ketoglutarate, respectively. It is conceivable, however, that in some pathological condition the metabolism of glial cells is impaired, leading to the accumulation of NH₄⁺ and glutamate and therefore to swelling in glia (Albrecht et al., 1994). This would lead to a deficient fuelling of neurons and possibly contribute to neuronal death.

This tight regulation of metabolic coupling between neurons and glial cells by means of chemical signals turns the nourishing of neurons by glia into a function, instead of a passive process, as it is widely considered (see Pfiuger and Barres, 1995). It is tempting to extrapolate some elements of the model presented in Figure 4 to the vertebrate nervous system, even if differences seem to exist. The Na⁺ dependence of glutamate uptake in glial cells, notably studied in salamander Müller glial cells, shares similar properties to that in the honeybee glial cells. The glutamate-induced increase in NAD(P)H fluorescence described here also was found in salamander Müller cells, initially under somewhat artificial conditions of voltage clamp (Barbour et al., 1993) and more recently in intact, acutely isolated Müller cells. Fluorescence image analysis revealed that this increase occurs in all parts of the cell, including the mitochondria-free end feet (Uga and Smelser, 1973), thus suggesting a glutamate-induced increase in glycolytic NADH (Tsacopoulos and MacLeish, 1996).

Astrogial energy metabolism is stimulated by increased Na⁺ entry, probably via a specific activation of the Na⁺ pump (Takahashi et al., 1995). Because glutamate in astroglia is cotransported with Na⁺, this would lead to an increased [Na⁺], and stimulation of glucose metabolism. Recent results of Pellerin and Magistretti (1994) are in accordance with this prediction. The activation of the pump is, however, an unlikely explanation for the metabolic effect of glutamate described in acutely isolated retinal glial cells, because aspartate uses the same transporter as glutamate (Christensen, 1990) but, in contrast to glutamate, does not induce an increase in NAD(P)H either in honeybee glial cells (see Results) or in salamander Müller cells (Bouvier et al., 1992). Honeybee glial cells play a crucial role in the positioning of sodium pumps on the plasma membrane of the photoreceptor and in retaining the pumps at the contact sites. They have, however, very few pumping sites on their own membranes (Baumann and Takeyasu, 1993). Indeed, pumping Na⁺ is not a crucial mechanism for spatial buffering of K⁺ by glia during neuronal stimulation in both the insect and vertebrate retina (Coles, 1989).

Apparently, the fixation of ammonia occurs differently in honeybee glial cells and astrocytes. In vertebrate nervous tissue, glutamine synthetase is localized predominantly within astrocytes (Norenberg and Martinez-Hernandez, 1979) and in Müller glial cells (Riepe and Norenberg, 1977), suggesting a predominant formation of glutamine there. Recently, Wani et al. (1992) found that ammonium at near-physiological concentrations (~100 μM) more than doubles the rate of glutamate to glutamine transformation in astrocyte cultures. This was evidence that the metabolism of glutamate to glutamine in astrocytes depends on extracellular ammonia. However, so that ammonia might be established as a metabolic signal in astrocytes, it is necessary to demonstrate that neuronal activity induces an increase of NH₄⁺ production that does not lead to saturation of the glutamate synthetase reaction occurring in astrocytes ($K_m$ for NH₄⁺ is ~0.18 mM; Palmiljans et al., 1962). Contrary to astrocytes, the acutely isolated honeybee glial cells do not synthesize glutamine but, instead, aminate pyruvate to alanine. Glutamine found in this retina probably is formed in the mitochondria of photoreceptors, a compartment in which the availability of ATP is likely to be higher than in the mitochondria-poor glial cells. This assumption is compatible with the finding in superfused slices of intact honeybee retina that 15N-glutamine is formed from 15N-proline, a substrate known to be consumed predominantly in the mitochondria of photoreceptors (Tsacopoulos et al., 1994, 1997). In any case, if there is a glutamate–glutamine cycle (see discussion by Yudkoff et al., 1992), nitrogen fixed on glutamine ultimately must return to astrocytes so that synthesis of amino acids can continue. Cultured astrocytes exposed to NH₄Cl for 4 d swell (Norenberg et al., 1991), possibly indicating the existence of some unidirectional NH₄⁺ transport system similar to the one we found in the honeybee glial cells.

Glutamate signaling between cortical astrocytes and neurons was shown recently to occur in mammalian cell culture (Parpura et al., 1994). However, the signaling that we have described in the present paper goes further, confirming the function of glial cells in nourishing and maintaining nervous tissue.

REFERENCES


