

Effect of Glucose Deprivation and Acute Glutamate Exposure in Cultured Retinal Cells

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The relationship between bioenergetics and the glutamate system was analyzed in a neuronal model of retinal cells in culture, submitted to glucose deprivation and exposed to glutamate for 2 h, and compared with exposure to glutamate in the presence of glucose. Under glucose deprivation, a reduction (about 1.1-fold) in the energy charge of the cells occurred, probably as a result of a decrement (by about 75%) in the cellular redox efficacy, as determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) test. In the absence of glucose, exposure of retinal cells to 10 μ M glutamate potentiated the reduction in the energy charge (by about 1.2-fold) and induced a significant increase in the uptake of $^{45}\text{Ca}^{2+}$ by the cells (1.3-fold), although no significant changes were observed in the presence of glucose. Under glucose deprivation, 100 μ M glutamate caused an irreversible cell membrane damage, as shown by the significant increase in lactate dehydrogenase (LDH) leakage (about 1.8-fold). A significant increase in membrane depolarization, measured by the reduction of [^3H]tetraphenylphosphonium⁺ (^3H]TPP⁺) uptake, was also observed after glutamate exposure in the absence of glucose. In the presence of glucose, high glutamate concentrations (10 mM) induced a major increase in Ca^{2+} entry into the cells and membrane depolarization, without affecting the energy charge or cell survival. In contrast, in the absence of glucose, 10 mM glutamate did not alter Ca^{2+} accumulation by the cells and a smaller decrease in membrane potential occurred, as compared to 100 μ M glutamate exposure. Data shown in this study suggest that during a prolonged (2 h) and acute exposure to high glutamate (10 mM), under glucose deprivation conditions, the neuronal systems have “adaptive” mechanisms that allow the survival of cells. These findings may have implications in neuronal degeneration occurring during brain ischemia. © 1998 Academic Press

Key Words: Ca^{2+} uptake; cell injury; energy charge; glucose deprivation; glutamate; membrane potential; retinal cells

INTRODUCTION

L-Glutamate and related excitatory amino acids (EAA) play an essential role in fast synaptic transmission and plasticity in the central nervous system, by interacting with glutamate receptors. The maintenance of glutamate extracellular concentrations below toxic levels is facilitated, in neuronal terminals or glial cells, by its reuptake via the high-affinity transport system. Otherwise, an excessive stimulation of ionotropic glutamate receptors induces the alteration of Ca^{2+} homeostasis (5,8), a proposed initial step for neuronal degeneration following ischemia, hypoglycemia, or trauma (7). Moreover, excitotoxicity, resulting from the dysfunction of the EAA system, is a mechanism thought to take place in several acute neurodegenerative diseases and finally leads to neuronal death. Previously, we showed that a decreased glucose metabolism, induced in the presence of iodoacetic acid or deoxyglucose, significantly decreased the adenylate energy charge in retinal cells in culture, used as neuronal models (19). Furthermore, *in vitro* conditions of hypoxia or ischemia in the retina system were shown to increase the release of glutamate (17, 19, 24), which seems to have a fundamental role in enhancing the neuronal damage.

Early work on glutamate toxicity has intensively focused on defining the contribution of ionotropic glutamate receptors (4, 5, 8), in order to search for good pharmacological tools that could prevent from glutamate excitotoxicity. During many neurodegenerative diseases and ischemia situations, glucose and/or oxygen deprivation combines with an excessive release of glutamate, followed by neurotoxic effects. Because excitotoxicity in the retina system has been focused on delayed neuronal damage (8, 20), in this study we analyzed some of the intracellular mechanisms associated with an acute exposure to glutamate under conditions of glucose deprivation in cultures of retinal cells, used as neuronal models. We focused both on low (10–100 μ M) glutamate concentrations, known to be involved with receptors activation, and high (10 mM) glutamate concentrations, suggested to occur locally under pathophysiological conditions, in the ischemic

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core or in the vicinity of a traumatic injury (12). For this purpose, we analyzed the glutamate-induced alterations of energy levels, Ca^{2+} uptake, and membrane potential, which were compared with changes in cell viability, determined by the release of LDH and the efficacy to reduce MTT. The present data show that under glucose deprivation, major glutamate-mediated cell injury is observed in the presence of low glutamate concentrations (10–100 μM), shown to be sufficient to activate the glutamate receptors, whereas in the presence of high glutamate concentrations (10 mM) neuronal damage is not further increased. The analysis of the cellular processes involved in neurodegeneration, during glutamate exposure, should give new insights on the excitotoxic neuronal death occurring under hypoglycemia or ischemia situations.

MATERIALS AND METHODS

Materials

Basal medium of Eagle (Earle's salts; BME) was purchased from Sigma Chemical Co. (St. Louis, Mo.), fetal calf serum was obtained from BioChrom KG (Berlin, Germany), and trypsin was from GIBCO (Paisley; UK). [^3H]Tetraphenylphosphonium $^+$ ([^3H]TPP $^+$; 23 Ci/mmol, as bromide salt) and $^{45}\text{Ca}^{2+}$ (13 mCi/mg calcium) were obtained from Amersham International (Amersham Centre, UK). All other chemicals used were purchased from Sigma Chemical Co.

Culture of Retinal Cells

Retinal cells were obtained from 8-day-old chick embryos. The retinas were trypsinized (0.1%) in Hank's medium without Ca^{2+} or Mg^{2+} , for 15 min, at 37°C, and then centrifuged at 500g, for 1 min (room temperature). The cells were resuspended in BME culture medium, supplemented with 5% fetal calf serum, 100 U/ml penicillin, and 100 mg/ml streptomycin, and buffered with 25 mM HEPES and 10 mM NaHCO_3 . Retinal cells were plated at a density of 0.44×10^6 cells/cm 2 on Costar 12-multiwell plates, previously coated with poly-L-lysine. The cells were maintained in culture for 5–6 days at 37°C in an atmosphere of 95% air and 5% CO_2 . A cell preparation similar to the one used in this work was shown to contain a significant percentage of amacrine-like neurons and neurons resembling bipolar cells and only a few glial cells (1, 10).

Incubation of Retinal Cells

Retinal cells were washed and incubated in Na^+ medium, containing (in mM): 140 NaCl, 5 KCl, 1.5 CaCl_2 , 1 MgCl_2 , 1 NaH_2PO_4 , 5.6 glucose, and 20 HEPES, at pH 7.4, 37°C, for 15 min. Glucose deprivation conditions were induced for 30 min (– glucose) before glutamate exposure. Other cells were incubated with

medium containing glucose (+ glucose). Then, L-glutamate (10 μM , 100 μM , or 10 mM) was incubated, respectively, in the absence or in the presence of glucose, for 2 h, at 37°C. Control retinal cells were incubated in the absence of glutamate.

Determination of Adenine Nucleotides

Retinal cells were extracted with 0.3 M perchloric acid right after the incubation with glutamate. Then, the acid extract was centrifuged at 15,800g, for 5 min, at 0–4°C. The resulting pellets were analyzed for total protein content (21), whereas the supernatants were neutralized with 10 M KOH in 5 M Tris. After neutralization the samples were centrifuged at 15,800g, for 10 min, at 0–4°C. The supernatants were assayed for ATP, ADP, and AMP, by separation in a reverse phase HPLC (23). The adenine nucleotides were eluted at a flow rate of 1.2 ml/min with 100 mM KH_2PO_4 , pH 7.4, and 1% methanol, and detected at 254 nm, for 6 min. The adenylate energy charge (EC) was calculated according to the formula (3):

$$\text{EC} = (\text{ATP} + 0.5 \text{ADP}) / (\text{ATP} + \text{ADP} + \text{AMP}).$$

$^{45}\text{Ca}^{2+}$ Uptake Measurements

$^{45}\text{Ca}^{2+}$ (1.5 $\mu\text{Ci/ml}$) uptake measurements were performed during the glutamate incubation, for 2 h (37°C), in the presence or in the absence of glucose. When present, 5 μM nitrendipine, a blocker of L-type Ca^{2+} -channels, was incubated for 30 min in the presence or in the absence of glucose and during the 2-h exposure to glutamate. The reaction was stopped by washing the cells twice with 1 mM LaCl_3 and once with 1 mM LaCl_3 and 1 mM EGTA (prepared in Na^+ solution without NaH_2PO_4 or CaCl_2). The cells were extracted with 0.1 M NaOH and the radioactivity of $^{45}\text{Ca}^{2+}$ retained by retinal cells was determined by scintillation spectroscopy and further expressed as dpm/ 10^6 cells.

[^3H]TPP $^+$ Uptake Measurements

[^3H]TPP $^+$ (0.25 $\mu\text{Ci/ml}$) was incubated after the treatment of retinal cells with glutamate. Incubations of [^3H]TPP $^+$ were performed in Na^+ or K^+ medium (Na^+ was isoosmotically substituted by KCl), for 15 min, just before the steady-state level of TPP $^+$ accumulation by retinal neurons in culture (2), at 37°C. The TPP $^+$ uptake reaction was terminated by adding 10% TCA (w/v). Then the cells were centrifuged at 15,800g, for 2 min, and the radioactivity of the supernatant was determined by scintillation spectroscopy. The membrane potential, only across the plasma membrane, was determined by calculating the difference in the uptake of [^3H]TPP $^+$ in Na^+ and K^+ medium (Na-K), because TPP $^+$ accumulation after depolarization of the plasma

membrane with 140 mM K⁺ mainly reflects the large negative mitochondrial membrane potential. The results were expressed as dpm/2 × 10⁶ cells.

Analysis of Cell Viability

Experiments were performed to monitor the leakage of LDH (lactate dehydrogenase) or to follow the reduction of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), which is based on the reduction capacity of mitochondrial dehydrogenases to reduce the MTT salt into formazan, an insoluble product (16).

LDH activity was measured spectrophotometrically by following the conversion of NADH to NAD⁺, at 340 nm, as described elsewhere (19). LDH leakage to the extracellular medium was expressed as a percentage of total LDH activity.

The MTT assay consisted in the incubation of 0.5 mg/ml MTT (0.5 ml) dissolved in Na⁺ medium in the absence or in the presence of glucose, during glutamate exposure, for 2 h. At the end of the incubation at 37°C, an equal volume of 0.04 M HCl in isopropanol was added, and the resulting mixture was recovered from the cell plates. The extent of MTT reduction was measured spectrophotometrically at 570 nm. Cell toxicity was expressed as a percentage of OD of control cells, in the absence of glutamate, but in the presence of glucose.

Data Analysis

The results are the means ± SEM of the indicated number of experiments. Statistical values ($P < 0.05$) were determined by the unpaired, two-tailed, Student's *t* test or by the one-way ANOVA with a Bonaferroni posttest, for multiple comparisons.

RESULTS

Effect of Glutamate on the Energy Charge of the Cells under Glucose Deprivation

The effect of glutamate, under glucose-free conditions, was analyzed by determining the energy charge in retinal cells in culture (Fig. 1). In the presence of glucose, low (10 or 100 μM) or high (10 mM) glutamate concentrations did not significantly alter the energy levels of the cells. However, in the absence of glucose, cells not submitted to glutamate exposure (controls) showed a significant reduction in the energy charge (0.81 ± 0.02 , $P < 0.01$), as compared to controls in the presence of glucose (0.89 ± 0.02). Under glucose deprivation, larger decrements in the energy charge (EC of approximately 0.65), indicative of a significant decrease in ATP (not shown), were observed in the presence of glutamate (10 μM and up to 10 mM). Under these conditions, the energy levels decreased significantly, as compared to the controls in the absence of

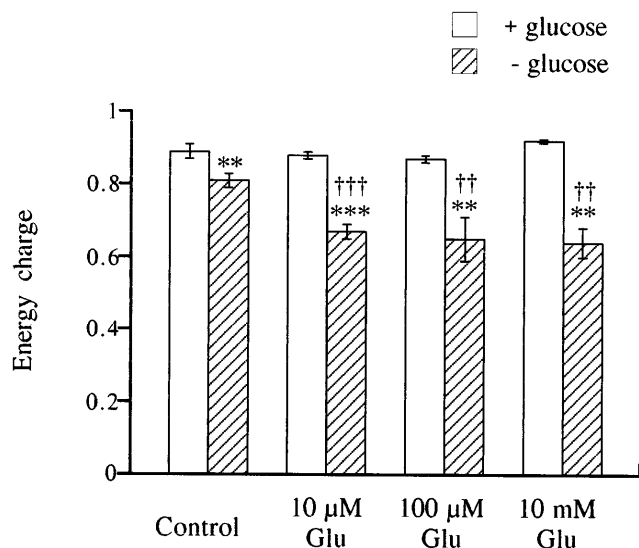


FIG. 1. Energy charge of retinal cells submitted to glutamate in the presence or in the absence of glucose. Adenylate energy charge was calculated by determining the intracellular levels of ATP, ADP, and AMP in retinal cells preincubated in the absence or in the presence of glucose for 30 min, and further exposed to glutamate (10 μM, 100 μM, or 10 mM) for 2 h, respectively, in glucose-free (–glucose) or glucose-containing (+ glucose) medium, at 37°C. Controls were incubated in the absence of glutamate. The data are the means ± SEM of duplicates or triplicates from two to three experiments. Statistical significance: ** $P < 0.01$ or *** $P < 0.001$ as compared to the control in the presence of glucose; †† $P < 0.01$ or ††† $P < 0.001$ as compared to the control in the absence of glucose.

glucose, indicating that exposure to glutamate potentiates the decrement of the energy charge caused by glucose deprivation (Fig. 1).

Intracellular Ca²⁺ Accumulation after Glutamate Exposure under Glucose Deprivation

In order to evaluate the intracellular mechanisms of glutamate injury that mediated the reduction in energy charge in retinal cells submitted to glucose deprivation, we studied the uptake of ⁴⁵Ca²⁺. In the presence of glucose, glutamate (100 μM or 10 mM) increased significantly the uptake of ⁴⁵Ca²⁺, as compared to the control (Fig. 2). Glucose deprivation, per se, did not significantly influence the entry of ⁴⁵Ca²⁺ in control retinal cells. However, exposure to 10 μM glutamate, in the absence of glucose, increased significantly the uptake of ⁴⁵Ca²⁺ (599.0 ± 30.0 dpm/10⁶ cells, $P < 0.001$), as compared to 10 μM glutamate in the presence of glucose (467.0 ± 18.2 dpm/10⁶ cells). In the absence of glucose, 100 μM glutamate (3094.7 ± 107.6 dpm/10⁶ cells) induced a maximal accumulation of ⁴⁵Ca²⁺. Thus, the ⁴⁵Ca²⁺ taken up after incubation with 10 mM glutamate, in the absence of glucose, was significantly lower ($P < 0.001$), as compared to retinal cells incubated with the same concentration of glutamate in the presence of glucose (Fig. 2). Nitrendipine (5 μM), a

blocker of L-type Ca^{2+} channels, which was shown to play a major role on $[Ca^{2+}]_i$ changes upon glutamate receptor activation in retinal cells in culture (6), did not significantly influence the intracellular accumulation of $^{45}Ca^{2+}$ after incubation with glutamate for 2 h, in the presence or in the absence of glucose (data not shown).

Alteration of Membrane Polarization after Glutamate Exposure in the Absence of Glucose

We also analyzed the alterations in membrane potential upon glutamate exposure, under normal or glucose deprivation conditions, by examining the uptake of $[^3H]TPP^+$ in retinal neurons in culture (Fig. 3). TPP^+ is a permeant lipophilic cation that easily permeates the membrane and accumulates in cells, allowing an indirect evaluation of alterations in membrane potential (13). In the presence of glucose, the plasma membrane potential, represented by the difference of $[^3H]TPP^+$ uptake observed in Na^+ and K^+ medium (Na-K), significantly decreased upon exposure to 10 mM glutamate. Control retinal cells submitted to glucose-free medium showed a large decrease in membrane potential (2737.0 ± 222.4 dpm/ 2×10^6 cells, $P < 0.01$), as compared to cells incubated in glucose medium (4434.1 ± 406.3 dpm/ 2×10^6 cells). Nevertheless, retinal neurons exposed to 100 μM or 10 mM glutamate, in the absence of glucose, showed a major membrane depolarization in comparison with controls under the same experimental conditions (Fig. 3). However,

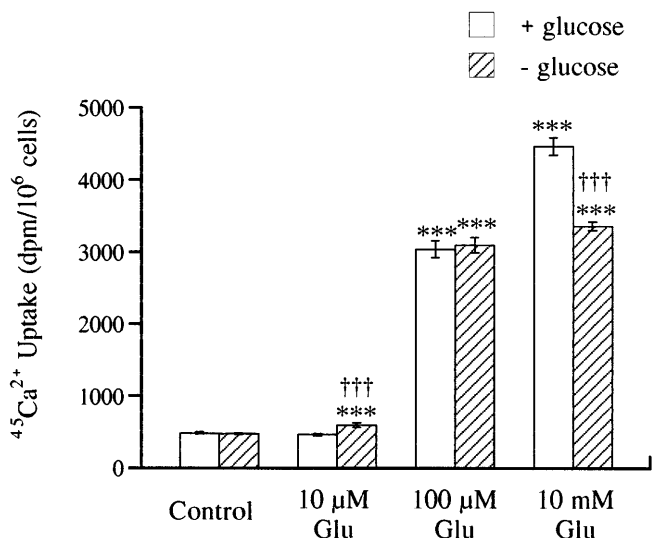


FIG. 2. Effect of glucose deprivation on the $^{45}Ca^{2+}$ uptake induced by glutamate. The $^{45}Ca^{2+}$ retained by retinal cells was measured upon glutamate (10 μM , 100 μM , or 10 mM) exposure for 2 h, in the presence (+ glucose) or in the absence (- glucose) of glucose. Control cells were incubated in the absence of glutamate. Data are the means \pm SEM of triplicates from three experiments. Statistical significance: *** $P < 0.001$ as compared to the respective controls; ††† $P < 0.001$ as compared to the respective data obtained in the presence of glucose, under similar experimental conditions.

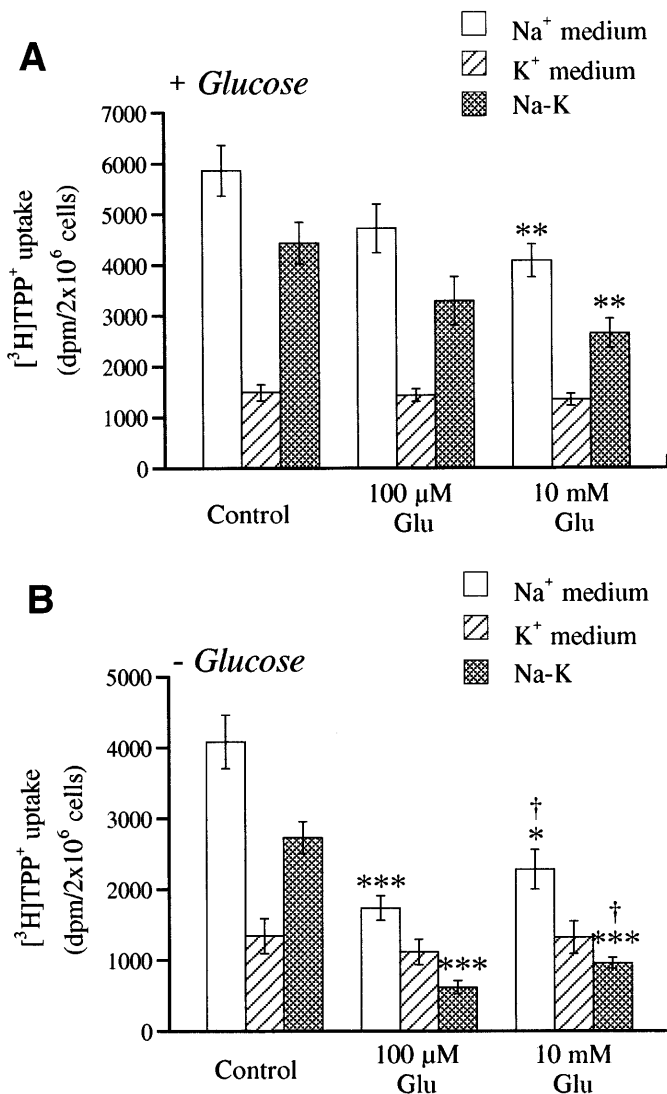


FIG. 3. Uptake of $[^3H]TPP^+$ upon glutamate exposure, in the presence or in the absence of glucose. Retinal cells were exposed to glutamate (10 μM , 100 μM , or 10 mM Glu) for 2 h in the presence (A) or in the absence (B) of glucose. $[^3H]TPP^+$ uptake measurements were determined in saline medium containing 140 mM NaCl (Na^+ medium) or containing 140 mM KCl (K^+ medium), for 15 min, after the incubation with glutamate. $[^3H]TPP^+$ uptake data indicated by Na-K represents the difference of data obtained in Na^+ and K^+ medium. The results are the means \pm SEM of duplicates from five experiments. Statistical significance: * $P < 0.05$, ** $P < 0.01$, or *** $P < .001$ as compared to the respective control; † $P < 0.05$ as compared to the respective data obtained in the presence of glucose.

10 mM glutamate exposure, in the absence of glucose, showed significantly higher ($P < 0.05$) TPP^+ uptake values, indicating an increase in membrane potential, as compared to 100 μM glutamate (Fig. 3).

Alteration of Cell Viability Following an Acute Exposure to Glutamate under Glucose Deprivation

Because exposure to glutamate under glucose deprivation was shown to cause significant changes in the

energy charge (Fig. 1) and in membrane potential (Fig. 3) of retinal cells in culture, we next examined the cell viability under similar experimental conditions, by measuring the leakage of LDH or the efficacy of retinal cells to reduce the MTT salt after glutamate exposure, for 2 h (Table 1).

Glucose deprivation, per se, or exposure to glutamate (up to 10 mM) in the presence of glucose did not cause a significant alteration in LDH leakage in comparison with control cells incubated with glucose. Under glucose-deprived conditions, exposure to 100 μ M glutamate significantly increased the release of LDH in comparison to controls (Table 1). Nevertheless, no further increments were observed upon exposure to 10 mM glutamate. The LDH leakage upon incubation with 100 μ M glutamate in glucose-free medium ($7.45 \pm 0.75\%$) was significantly higher ($P < 0.001$) as compared to the effect of glutamate in the presence of glucose ($3.66 \pm 0.37\%$). The results indicated that an acute exposure to glutamate, under glucose deprivation, caused some irreversible cell membrane damage.

Analysis of cell viability by the MTT method indicated that glucose deprivation, per se, decreased significantly ($25.1 \pm 1.8\%$ of control) the efficacy of retinal cells to reduce MTT (Table 1). These results indicate a major decrease of redox efficiency of retinal cells, probably related to mitochondrial functional deficits in the absence of glucose, which was not further affected upon exposure to glutamate.

DISCUSSION

This study shows that an acute exposure of retinal cells in culture to glutamate, under glucose-deprived conditions, causes significant intracellular alterations in cellular energy charge, intracellular Ca^{2+} accumulation, and membrane potential. Glucose deprivation alone induced a small decrease in the energy charge (Fig. 1) and a decrement in the cellular redox efficacy,

probably related with changes in mitochondrial function (11, 16), as determined by the MTT method (Table 1). The alteration of cellular redox state may have influenced the reduction in energy charge after incubation with glutamate, because exposure to glutamate potentiated the loss of energy levels in cells deprived of glucose (Fig. 1). A decrement in the energy charge in the absence of glucose may have also influenced the plasma membrane potential (Fig. 3), which could result from changes in membrane ionic fluxes. Previously, inducers of hypoglycemia-like conditions, such as the iodoacetic acid or deoxyglucose, were shown to significantly decrease the energy charge in cocultures of retinal cells (19). In the retina, Zeevalk and Nicklas (24) have also demonstrated that iodoacetic acid significantly decreases intracellular ATP, phosphocreatine, and lactate levels.

Under glucose-free conditions, exposure to 100 μ M glutamate, for 2 h, increased significantly the leakage of LDH (Table 1), indicating a decrease of plasma membrane integrity, which may be related with changes in energy charge, membrane depolarization, and Ca^{2+} uptake. Other authors have previously found that metabolic inhibitors predispose the neurons to glutamate toxicity (18, 22, 25). The excitotoxicity induced by glutamate, either mediated through the activation of the *N*-methyl-D-aspartate (NMDA) receptor (5) or the non-NMDA receptors (4), has been associated with the accumulation of Ca^{2+} in the cells. Our data showed that there is an increase in the uptake of Ca^{2+} induced by glutamate in the presence of glucose (100 μ M glutamate increased $^{45}\text{Ca}^{2+}$ uptake by about sixfold as compared to the control, as shown in Fig. 2), suggesting that a large elevation of intracellular Ca^{2+} does not necessarily predict a major decrease in cell viability, since we did not observe significant changes in LDH leakage (Table 1), an indicator of irreversible membrane damage. In the absence of glucose, 10 μ M glutamate increased significantly the uptake of Ca^{2+} , as

TABLE 1

Effect of Glucose Deprivation and Glutamate-Mediated Changes in Retinal Cells Viability

	+Glucose				-Glucose			
	Control	10 μ M Glu	100 μ M Glu	10 mM Glu	Control	10 μ M Glu	100 μ M Glu	10 mM Glu
LDH leakage (% of total)	3.01 ± 0.30	4.08 ± 0.79	3.66 ± 0.37	4.67 ± 0.44	4.12 ± 0.41	5.97 ± 0.46	$7.45 \pm 0.75^{***\dagger\dagger\dagger}$	$6.99 \pm 0.70^{**}$
MTT reduction (% of control)	100.0 ± 2.3	$87.9 \pm 0.5^*$	$82.7 \pm 2.5^{**}$	90.9 ± 3.2	$25.1 \pm 1.8^{\dagger\dagger\dagger}$	$28.8 \pm 1.1^{\dagger\dagger\dagger}$	$27.2 \pm 1.9^{\dagger\dagger\dagger}$	$28.8 \pm 2.3^{\dagger\dagger\dagger}$

Note. Retinal cells were incubated in the presence or in the absence of glucose for 30 min and further exposed to glutamate (10 μ M, 100 μ M, or 10 mM) for 2 h with glucose (+glucose) or in glucose-free medium (-glucose), respectively. Cell viability was determined by measuring the leakage of LDH (in % of total) or the efficacy of retinal cells to reduce the MTT salt (in % of control, in the presence of glucose). The results are the means \pm SEM of duplicates or quadruplicates from six (LDH leakage) or three (MTT reduction) experiments. Statistical significance: * $P < 0.05$, ** $P < 0.01$, or *** $P < 0.001$ as compared to the controls in the presence or in the absence of glucose; $\dagger\dagger\dagger$ $P < 0.001$ as compared to the respective experimental condition observed in the presence of glucose.

compared to experimental conditions in the presence of glucose (Fig. 2). However, no significant differences were observed in cells treated with 100 μM glutamate in the presence or in the absence of glucose (Fig. 2). Because hypoglycemia-like conditions induced in the presence of iodoacetic acid, for 15 min, increased the extracellular accumulation of endogenous glutamate (not shown), an alteration in Ca^{2+} uptake, and cell viability in the presence of low glutamate concentrations might have been conditioned by the releasable pool of the amino acid. Nonetheless, the observation that glucose deprivation, per se, did not affect the uptake of Ca^{2+} suggests that, after a period of 2 h, the accumulated Ca^{2+} , resulting from activation of ionotropic glutamate receptors by endogenous glutamate, could have been extruded out of the cells. Low (100 μM) glutamate concentrations, in the absence of glucose, significantly decreased the membrane potential in retinal cells in culture (Fig. 3), probably resulting from the decrement in energy levels (Fig. 1). Low concentrations of glutamate were also found to preferentially activate the NMDA receptors (5), which may lose the voltage-dependent Mg^{2+} blockade during metabolic inhibition (25), thus contributing to enhance receptor activation (18). These results suggest that under glucose deprivation, one of the primary events to induce excitotoxicity, in the presence of low glutamate concentrations, involves a decrement in energy charge, associated with a decreased redox state of the cells and, eventually, with a decrease integrity of the membrane (Table 1). Because we could not find significant decrements in the glutamate-induced uptake of Ca^{2+} in the presence of nitrendipine (5 μM), a blocker of the L-type voltage-sensitive Ca^{2+} -channels (not shown), the increments in Ca^{2+} accumulation may have resulted from the entry of Ca^{2+} through the glutamate receptor-associated channels. Ferreira *et al.* (8) have previously found that the permeation of Ca^{2+} through the glutamate receptors, and specially that occurring through the NMDA receptor for small Ca^{2+} loads, correlated well with a subsequent increase in retinal delayed cell death, as determined by the MTT method upon 18–20 h of glutamate exposure. A decrease in the redox state of retinal cells submitted to metabolic inhibition by induction of chemical ischemia, previously shown to increase the release of endogenous glutamate (19), resulted partially from the activation of AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate)/kainate glutamate receptors (9). In the isolated retina, glutamate, NMDA, AMPA, and kainate were all shown to cause delayed, as well as acute excitotoxic injury (20). Several studies demonstrated a decrease of cell death by using antagonists of NMDA or non-NMDA glutamate receptors, either when cell damage was induced by glutamate exposure (8, 14) or by metabolic inhibition (15, 24).

By testing 10 mM glutamate, a high extracellular

glutamate concentration, expected to occur after cell disruption, when the intracellular constituents gain access to the interstitial space under pathophysiological conditions (12), we showed that neither the energy charge nor the cell viability was affected, as compared to the results observed in the presence of low glutamate concentrations. Nevertheless, the uptake of Ca^{2+} , after exposure of retinal cells to 10 mM glutamate in the absence of glucose, was significantly lower as compared to cells incubated in the presence of glucose (Fig. 2). The observation that maximal Ca^{2+} accumulation occurred after exposure to 100 μM glutamate under glucose deprivation suggested that a partial reduction of cellular energy levels (Fig. 1) may have influenced a bulk entry of Ca^{2+} . Incubation with 10 mM glutamate, in the absence of glucose, also induced a significant increase in TPP^+ uptake as compared to 100 μM glutamate (Fig. 3). Thus, a small, but significant, decrease in membrane depolarization after 10 mM glutamate exposure, under glucose-deprived conditions, could partly explain a decrement in Ca^{2+} accumulation. We may also consider that the entry of Ca^{2+} occurring through the glutamate receptors may have decreased in the presence of high glutamate concentrations (10 mM), because NMDA receptor activation depends on cell depolarization.

In conclusion, we demonstrate that glucose deprivation and a decrement in energy levels, probably associated with a decrement in mitochondrial function, predispose retinal neurons to glutamate injury after an acute exposure. Glucose deprivation induced a reduction in the energy charge and a decrease in membrane potential, which may be responsible for an increase in Ca^{2+} entry into the cells in the presence of low glutamate. Interestingly, the present data also suggest the possibility that retinal neurons may have "adaptive" mechanisms to overcome excitotoxic effects induced by high extracellular glutamate concentrations, because in cells exposed to glucose deprivation, the decrease in the energy charge or the accumulation of Ca^{2+} were not enhanced upon addition of 10 mM glutamate, as compared to 100 μM glutamate. Furthermore, membrane depolarization decreased in cells exposed to 10 mM glutamate under glucose deprivation, which could explain a maintenance of Ca^{2+} accumulation, as compared to 100 μM glutamate. These data also suggest the possibility of a decreased activation of NMDA glutamate receptors, thus avoiding major intracellular changes. Although not reflecting changes occurring on delayed retinal cell death, the present results may be important in determining the intracellular mechanisms associated with glutamate-mediated neuronal injury that occur under acute hypoglycemia or metabolic compromised conditions, which can predispose to further cell degeneration.

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