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# Glutamate uptake into astrocytes stimulates aerobic glycolysis: A mechanism coupling neuronal activity to glucose utilization

(glutamate transporter/$Na^+/K^+$-ATPase/2-deoxyglucose/positron-emission tomography/magnetic resonance imaging)

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**ABSTRACT** **Glutamate, released at a majority of excitatory synapses in the central nervous system, depolarizes neurons by acting at specific receptors. Its action is terminated by removal from the synaptic cleft mostly via $Na^+$-dependent uptake systems located on both neurons and astrocytes. Here we report that glutamate, in addition to its receptor-mediated actions on neuronal excitability, stimulates glycolysis—i.e., glucose utilization and lactate production—in astrocytes. This metabolic action is mediated by activation of a $Na^+$-dependent uptake system and not by interaction with receptors. The mechanism involves the $Na^+/K^+$-ATPase, which is activated by an increase in the intracellular concentration of $Na^+$ cotransported with glutamate by the electrogenic uptake system. Thus, when glutamate is released from active synapses and taken up by astrocytes, the newly identified signaling pathway described here would provide a simple and direct mechanism to tightly couple neuronal activity to glucose utilization. In addition, glutamate-stimulated glycolysis is consistent with data obtained from functional brain imaging studies indicating local nonoxidative glucose utilization during physiological activation.**

Glutamate, the main excitatory neurotransmitter in the brain, profoundly affects neuronal activity by interacting with specific ionotropic and metabotropic receptors (1). The postsynaptic actions of glutamate are rapidly terminated by avid reuptake systems located on both neurons and astrocytes surrounding the synaptic cleft. Both neuronal and astrocytic glutamate transporters have been cloned and their properties studied *in vitro* (2). In astrocytes, the major glutamate transport is an electrogenic process by which one glutamate is cotransported with three $Na^+$ (or two $Na^+$ and one $H^+$) in exchange for one $K^+$ and one $OH^-$ (or one $HCO_3^-$) (3). The consequence of this stoichiometry is an increase in the $Na^+$ concentration within the astrocyte, accompanied by an intracellular acidification and extracellular alkalinization. Glutamate uptake is essential not only to terminate its effects as neurotransmitter, but also to prevent extracellular glutamate levels from reaching excitotoxic levels (4). In this study, we report that glutamate uptake into astrocytes also results in the stimulation of glucose utilization and lactate production. This metabolic action of glutamate, via a newly identified signaling mechanism, provides a simple and straightforward explanation for the coupling existing between neuronal activity and glucose utilization as observed both in animal experiments (5, 6) and *in vivo* in humans (7).

## MATERIALS AND METHODS

2-Deoxy-D-[1,2-$^3$H]glucose ([$^3$H]2DG) was purchased from DuPont (specific activity, 30.6 Ci/mmol; 1 Ci = 37 GBq). D(−)-2-Amino-5-phosphonopentanoic acid, 6-cyano-7-nitroquinoxaline-2,3-dione, L(+)-2-amino-3-phosphonopropionic acid, L(+)-2-amino-4-phosphonobutyric acid, and (2*S*,3*S*,4*R*)-α-(carboxycyclopropyl)glycine (L-CCG III) were obtained from Tocris Neuramin (Bristol, U.K.). Fetal calf serum was purchased from Seromed (Berlin), while Dulbecco's modified Eagle's medium (DMEM) and all other chemicals were from Sigma.

**Preparation of Primary Cultures of Mouse Cerebral Cortical Astrocytes.** Primary cultures of cerebral cortical astrocytes were prepared from Swiss albino newborn mice (1–2 days old) as described (8, 9). This procedure yields cultures that are >95% immunoreactive for glial fibrillary acidic protein (8, 9).

**[$^3$H]2DG Uptake.** Primary cultures of cerebral cortical astrocytes were used at confluence, usually between 19 and 22 days after seeding. [$^3$H]2DG uptake was determined as described (9). On the day of the experiment, the culture medium was replaced by serum-free DMEM (cat. no. D5030) supplemented with 5 mM glucose, 44 mM $NaHCO_3$, 0.06 g of penicillin per liter, 0.1 g of streptomycin per liter, and 0.045 mM phenol red ($DMEM_5$). Cells were incubated for 2 hr at 37°C in a water-saturated atmosphere containing 5% $CO_2$/95% air. The medium was then replaced by 2 ml of the same $DMEM_5$ medium containing [$^3$H]2DG at a concentration of 1 μCi/ml (33 nM). Pharmacological agents were added as 20-μl aliquots. Antagonists or inhibitors were added 20 min before addition of [$^3$H]2DG and maintained throughout the incubation. Agonists were added immediately after [$^3$H]2DG and the cells were further incubated for 20 min in the same conditions as previously indicated. The reaction was stopped by aspiration of the medium, cells were rinsed three times with ice-cold phosphate-buffered saline, and 2 ml of 0.1 M NaOH/0.1% Triton X-100 was added to lyse the cells. Aliquots of 500 μl were assayed for radioactivity by liquid scintillation counting, while 50-μl aliquots were used for measurement of protein by the method of Bradford (10). Results, which represent glucose transporter-mediated uptake and subsequent phosphorylation, were calculated by subtracting from total counts the portion that was not inhibited by the glucose transporter inhibitor cytochalasin B (10 μM). The cytochalasin-sensitive uptake accounted for ≈80% of total uptake (9).

**Lactate Release.** Lactate release into the medium was measured enzymatically by a modification of the enzymatic–spectrophotometric method of Rosenberg and Rush (11). Incubations were carried out exactly as described for [$^3$H]2DG uptake experiments except for the fact that no tracer and no phenol red (which otherwise interferes with the spectrophotometric determination of lactate) were present in the incubation medium. The reaction was terminated by collecting the supernatant on ice, while cells were treated as described above for protein determination.

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Abbreviations: [$^3$H]2DG, 2-deoxy-D-[1,2-$^3$H]glucose; L-CCG III, (2*S*,3*S*,4*R*)-α-(carboxycyclopropyl)glycine; THA, DL-*threo*-β-hydroxyaspartate; $[Na^+]_i$, intracellular $[Na^+]$.

## RESULTS

Basal glucose utilization by primary cultures of astrocytes prepared from mouse cerebral cortex was monitored with [$^3$H]2DG as described (9). Considering the specific activity of [$^3$H]2DG, glucose utilization by astrocytes ranges between 4 and 9 nmol per mg of protein per min, a set of values of the same order as the one determined for rodent cortical gray matter with the 2DG autoradiography method (between 10 and 16 nmol per mg of protein per min if one assumes a protein content for the brain of 10%) (5). L-Glutamate stimulates 2DG uptake and phosphorylation by astrocytes in a concentration-dependent manner, with an $EC_{50}$ of 80 $\mu$M (Fig. 1). This effect is not mediated by specific glutamate receptors known to be present on astrocytes (12), since it was not inhibited by any of the specific antagonists tested (Table 1). Likewise, agonists specific for each receptor subtype such as *N*-methyl-D-aspartate, DL-$\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, quisqualate, or (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid did not mimic the effect of glutamate on [$^3$H]2DG uptake and phosphorylation at concentrations up to 500 $\mu$M (data not shown). Only homocysteate and kainate exhibited a modest effect (54% and 24% increase, respectively, at 500 $\mu$M). The effect of glutamate was stereospecific, with only the L isomer being active, while both D- and L-aspartate exhibited an action comparable to that of L-glutamate (Fig. 2*A*). This stereospecificity profile is characteristic of the glutamate transporter (13). Consistent with this view, the uptake and phosphorylation of [$^3$H]2DG evoked by glutamate were completely abolished by the potent glutamate transporter inhibitor DL-*threo*-$\beta$-hydroxyaspartate (THA) as illustrated in Fig. 2*B*; a similar observation was made with another glutamate uptake inhibitor, L-CCG III (Fig. 2*B*). As noted earlier, glutamate uptake into astrocytes is $Na^+$ dependent; accordingly, replacement of $Na^+$ in the medium completely abolished the stimulation of [$^3$H]2DG uptake and phosphorylation evoked by glutamate (Fig. 2*C*). These results demonstrate a tight coupling between $Na^+$-dependent glutamate uptake and glucose utilization by astrocytes.

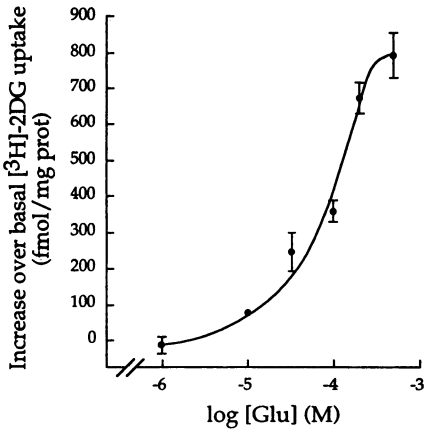


FIG. 1. Concentration–response curve of the stimulation by glutamate of [$^3$H]2DG uptake by astrocytes. Accumulation of [$^3$H]2DG in primary cultures of mouse cerebral cortical astrocytes was measured after a 20-min incubation in the presence of increasing concentrations of L-glutamate. Basal [$^3$H]2DG uptake was 590 ± 44 fmol per mg of protein per 20 min ($n$ = 3), corresponding to an uptake of 4.5 nmol of glucose per mg of protein per min calculated from the specific activity of [$^3$H]2DG. An $EC_{50}$ value of 80 ± 24 $\mu$M ($n$ = 3 separate experiments) was obtained. Results are means ± SEM of triplicate determinations from one experiment repeated twice with similar results. The effect of glutamate at 100, 200, and 500 $\mu$M was determined in at least five separate experiments for each concentration with similar results.

Table 1. Effect of glutamate receptor antagonists on glutamate-induced increase in [$^3$H]2DG uptake by astrocytes

| Treatment | [$^3$H]2DG uptake, fmol per mg of protein |
|---|---|
| None | 871 ± 35 |
| Glutamate (500 $\mu$M) | 1581 ± 45 |
| + D-AP5 (1 mM) | 1512 ± 22 |
| + CNQX (0.1 mM) | 1671 ± 23 |
| + L-AP3 (1 mM) | 1514 ± 50 |
| + L-AP4 (1 mM) | 1493 ± 38 |

Results are means ± SEM of at least three separate determinations. Incubation was 20 min. D-AP5, D(−)-2-amino-5-phosphonopentanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; L-AP3, L(+)-2-amino-3-phosphonopropionic acid; L-AP4, L(+)-2-amino-4-phosphonobutyric acid.

A previous study had indicated that increasing intracellular $Na^+$ concentration ($[Na^+]_i$) with the ionophore monensin resulted in a marked stimulation of [$^3$H]2DG uptake and phosphorylation by astrocytes, which was inhibited by ouabain (14), thus suggesting a functional link between $Na^+/K^+$-ATPase activity and glucose utilization. Accordingly, as shown in Fig. 3, the [$^3$H]2DG uptake and phosphorylation activated by $Na^+$-dependent glutamate uptake was completely inhibited by ouabain. The astrocytic $Na^+/K^+$-ATPase responds predominantly to increases in $[Na^+]_i$ for which it shows a $K_m$ of ≈10 mM (15). Since in cultured astrocytes the $[Na^+]_i$ ranges between 10 and 20 mM (16), $Na^+/K^+$-ATPase is set to be readily activated when $[Na^+]_i$ increases concomitantly with glutamate uptake (17). In this context, it is important to note that *in vivo* the main mechanism that accounts for the activation-induced 2DG uptake is represented by the activity of the $Na^+/K^+$-ATPase (6). In addition, there is ample evidence from the literature indicating that, in a variety of cellular systems including the brain, kidney, vascular smooth muscle, and erythrocytes, increases in $Na^+/K^+$-ATPase activity stimulate glucose uptake and glycolysis (18–21). The molecular mechanism of this tight coupling has been suggested to reside in the close association between $Na^+/K^+$-ATPase and certain key enzymes of glycolysis in the plasma membrane, implying that glycolytically generated ATP, notably at the phosphoglycerate kinase step, is preferentially used for the activity of the pump (21, 22). From the foregoing we set out to examine whether aerobic glycolysis, as determined by the production of lactate, was stimulated by glutamate. The basal rate of lactate release by astrocyte cultures is high, ranging between 30 and 60 nmol per mg of protein per min (23), indicating the presence in astrocytes, as in a few other cell types (19–21), of active aerobic glycolysis. This glycolytic flux in astrocytes can be modulated, as indicated by the fact that inhibition of oxidative phosphorylation by azide results in a 3-fold increase in lactate release (24). As indicated in Fig. 4, glutamate markedly stimulated lactate release which, like glucose uptake, was completely abolished by THA, strongly suggesting that glutamate uptake triggers both metabolic responses. Astrocytes also released pyruvate and showed increased release upon stimulation with glutamate; basal pyruvate release is nonetheless ≈3 times lower than basal lactate release [basal pyruvate release, 267.6 ± 6.5 nmol per mg of protein per 30 min ($n$ = 3); glutamate stimulated (200 $\mu$M), 428.1 ± 15.5 nmol per mg of protein per 30 min ($n$ = 3)]. Glutamate-induced pyruvate release was also blocked by THA [basal pyruvate release with 1 mM THA, 217.7 ± 10.4 nmol per mg of protein per 30 min ($n$ = 3); glutamate stimulated (200 $\mu$M) with 1 mM THA, 229.8 ± 14.5 nmol per mg of protein per 30 min ($n$ = 3)]. Finally, the effect of glutamate on lactate release was blocked when the glucose transporter inhibitor cytochalasin B was applied (Fig. 4), indicating that glutamate

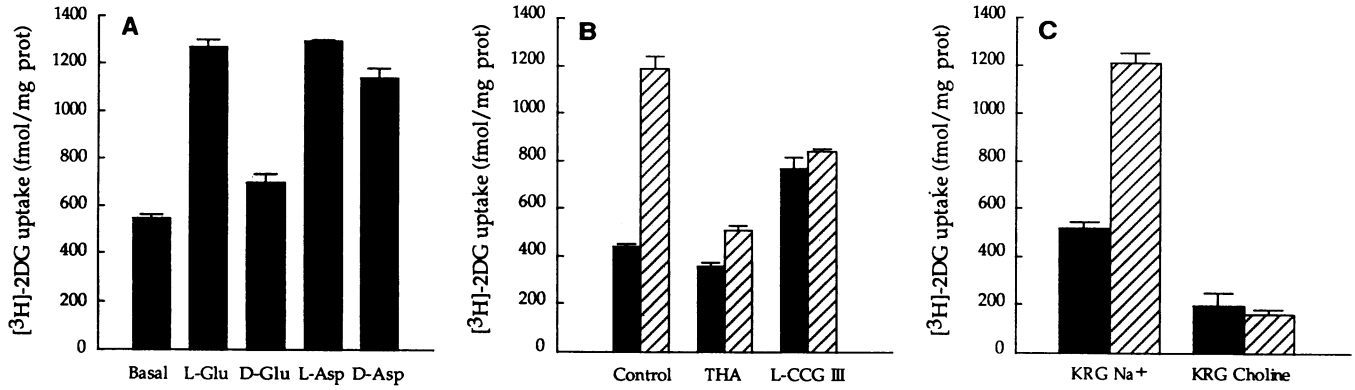


FIG. 2. (*A*) Stereospecificity of glutamate-induced increase in [$^3$H]2DG uptake. Astrocytes were exposed to each stereoisomer (200 $\mu$M) for a 20-min incubation, at the end of which the amount of [$^3$H]2DG uptake was determined. Results are means ± SEM of three separate determinations from one experiment repeated once with similar results. (*B*) Effect of glutamate transport inhibitors on glutamate-induced increase in [$^3$H]2DG uptake. The amount of [$^3$H]2DG uptake after 20 min was measured in the absence (solid bars) or in the presence (hatched bars) of 200 $\mu$M glutamate. Inhibitors were added at a concentration of 500 $\mu$M 20 min before glutamate. Results are means ± SEM of three separate determinations from one experiment repeated twice for THA with similar results. (*C*) Effect of $Na^+$ replacement on glutamate-induced increase in [$^3$H]2DG uptake. Cells were incubated in a normal Krebs–Ringer glucose (KRG) medium or in a $Na^+$-free KRG, with the following composition: normal KRG, 4.465 mM KCl, 1.8 mM $CaCl_2$, 0.833 mM $MgSO_4$, 0.9083 mM $KH_2PO_4$, 5 mM glucose, 0.045 mM phenol red, 109.5 mM NaCl, 44 mM $NaHCO_3$ (pH 7.4); $Na^+$-free KRG; NaCl and $NaHCO_3$ were replaced by choline chloride (109.5 mM) and choline bicarbonate (44 mM), respectively. [$^3$H]2DG uptake was determined after a 20-min incubation in each medium in the absence (solid bars) or in the presence (hatched bars) of 500 $\mu$M glutamate. Results are means ± SEM of three separate determinations from one experiment repeated once with similar results.

promotes both the uptake of glucose and its metabolism into lactate or, in other words, aerobic glycolysis.

## DISCUSSION

Data reported in this article indicate that glutamate, in addition to its receptor-mediated effects on neuronal excitability, also exerts a metabolic function in astrocytes that is mediated by a $Na^+$-dependent transport system in this cell type. Astrocytes are ideally suited to be the cellular locus of glutamate-induced glucose uptake and utilization. Thus, astrocytic end feet surround intraparenchymal capillaries, which represent the source of glucose (25). This cytoarchitectural arrangement implies that astrocytes form the first cellular barrier that glucose entering the brain parenchyma encounters, and it makes them a likely site of prevalent glucose uptake. In this context, it is interesting to consider the results obtained by Tsacopoulos and colleagues (26) in the honeybee drone retina. In this highly organized nervous tissue preparation, photoreceptor cells form rosette-like structures, which are surrounded by glial cells. Upon activation of the photoreceptors by light, an increase in [$^3$H]2DG uptake can be visualized in the glial cells surrounding the rosettes but not in the photoreceptors (26). An increase in $O_2$ consumption is nonetheless measured in photoreceptors. These experiments suggest that after activation of photoreceptors by light, glucose is predominantly taken up by glial cells, which then release a metabolic substrate to be oxidized by photoreceptor cells. Similar results have been reported by the same group in guinea pig retina (27).

All synapses, including glutamatergic ones, are ensheathed by astrocytic processes (25, 28) where glutamate uptake predominantly takes place (29). In fact, it is well established that activation-induced increase in 2DG uptake occurs in the neuropil—i.e., in regions enriched in axon terminals, dendrites, and synapses ensheathed by astrocyte processes—and not where neuronal perikarya are located. A striking demonstration of this selective localization of 2DG uptake

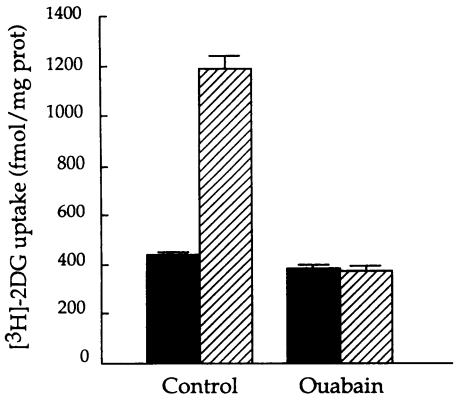


FIG. 3. Inhibition by ouabain of glutamate-induced increase in [$^3$H]2DG uptake. Cells were first exposed to 100 $\mu$M ouabain for 20 min. [$^3$H]2DG uptake was then determined over a 20-min incubation in the presence of 100 $\mu$M ouabain either without (solid bars) or with (hatched bars) 200 $\mu$M glutamate. Results are means ± SEM of three separate determinations from one experiment repeated once with similar results.

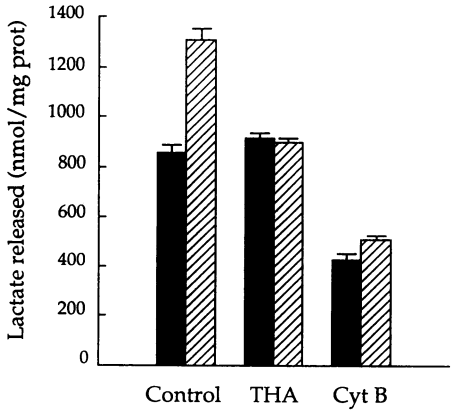


FIG. 4. Stimulation of lactate release by glutamate. Cells were incubated for 30 min in the absence (solid bars) or presence (hatched bars) of 200 $\mu$M glutamate. Either 1 mM THA or 10 $\mu$M cytochalasin B (Cyt B) was added 20 min before glutamate and maintained throughout the incubation. Lactate released in the medium was measured enzymatically. Results are means ± SEM of three separate determinations from one experiment repeated three times for glutamate and once for THA with similar results.

was provided in experiments by Sokoloff and colleagues (30). Electrical stimulation of the sciatic nerve in rats causes a frequency-dependent increase in 2DG uptake in the dorsal horn of the spinal cord (where afferent axon terminals make synaptic contacts with second-order neurons) but not in the dorsal root ganglion, where the cell body of the sensory neurons is localized. Furthermore, in monkey, increases in 2DG uptake in the well-laminated primary visual cortex elicited by appropriate visual stimuli are most pronounced in layer IV, which is poor in perikarya but where the terminals of axons projecting from the lateral geniculate engage in synaptic contacts (31).

Glucose taken up by astrocytes through the action of glutamate is metabolized glycolytically, as indicated by increased lactate production (Fig. 4). Recently, a specific transport system for lactate has been described in neurons, suggesting that lactate may represent an adequate metabolic substrate for this cell type (32). There is in fact evidence, accumulated over the years, that synaptic activity can be maintained in slices of cerebral cortex in the absence of glucose, with lactate or pyruvate as the sole energy substrates (33, 34). In addition, neurodegeneration in the hippocampal slice preparation induced by glucose deprivation is prevented by inclusion of lactate in the perfusing medium (35). Even in the presence of glucose, lactate utilization by nervous tissue has been demonstrated *in vitro* (36). In summary, while plasma lactate cannot fully substitute for glucose as a metabolic substrate for brain because of its limited permeability across the blood–brain barrier (37), lactate formed within the brain parenchyma—e.g., through glutamate-activated glycolysis in astrocytes—could fulfill at least in part the energetic needs of neurons during activation. Indeed lactate, after conversion to pyruvate via a reaction catalyzed by lactate dehydrogenase, can provide on a molar basis 18 ATPs through oxidative phosphorylation. Interestingly, a similar metabolic exchange between cell types has been described in the testis (38). In this case, active glycolysis in Sertoli cells produces lactate, which is the preferred metabolic substrate for round spermatids.

The results reported here are summarized in the model of cell-specific metabolic regulation, which is illustrated in Fig. 5. This model, which summarizes *in vitro* experimental evidence indicating glutamate-induced glycolysis, is taken to reflect cellular and molecular events occurring during activation of a given cortical area. Direct neuronal glucose uptake could still take place under these conditions, as it does in the basal state. It should also be noted that a reciprocal relationship appears to exist between aerobic glycolysis and glutamate uptake. Thus, glutamate uptake into astrocytes is markedly decreased by inhibition of glycolysis while being only moderately affected by hypoxia (39). These results suggest the existence of a cooperative mechanism whereby glutamate uptake triggers aerobic glycolysis, which in turn is necessary to maintain proper transmembrane glutamate and $Na^+$ gradients to direct glutamate transport into astrocytes.

The data reported here demonstrating glutamate-induced glycolysis in astrocytes may provide a cellular and molecular basis to explain the activation-induced glycolysis that is observed with various functional brain imaging techniques. Thus, using $^1$HNMR spectroscopy, increases in lactate signal are detected in primary visual cortex after physiological activation (40, 41). In addition, biochemical and *in vivo* microdialysis studies have revealed increased lactate levels in rat somatosensory cortex and hippocampus after physiological stimulation (42, 43). Positron-emission tomography studies in which blood flow, oxygen consumption, and glu-

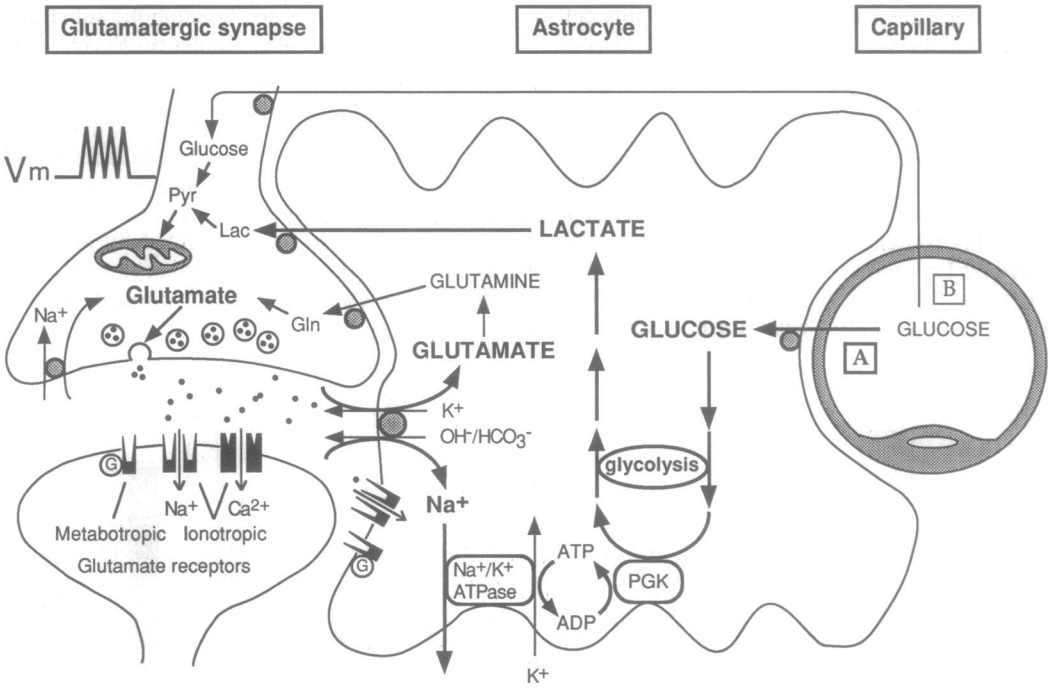


FIG. 5. Schematic of the mechanism for glutamate-induced glycolysis in astrocytes during physiological activation. At glutamatergic synapses, glutamate depolarizes neurons by acting at specific receptor subtypes. The action of glutamate is terminated by an efficient glutamate uptake system located primarily in astrocytes. Glutamate is cotransported with $Na^+$, resulting in an increase in $[Na^+]_i$, leading to activation of $Na^+/K^+$-ATPase. The pump, fueled by ATP provided by membrane-bound glycolytic enzymes [possibly phosphoglycerate kinase (PGK); see ref. 22], activates glycolysis—i.e., glucose utilization and lactate production—in astrocytes. Lactate, once released, can be taken up by neurons and serve as an adequate energy substrate. For graphic clarity, only lactate uptake into presynaptic terminals is indicated. However, this process could also occur at the postsynaptic neuron. Based on recent evidence, glutamate receptors are also shown on astrocytes (12). This model, which summarizes *in vitro* experimental evidence indicating glutamate-induced glycolysis, is taken to reflect cellular and molecular events occurring during activation of a given cortical area [arrow labeled A (activation)]. Direct glucose uptake into neurons under basal conditions is also shown [arrow labeled B (basal conditions)]. Pyr, pyruvate; Lac, lactate; Gln, glutamine; G, guanine nucleotide binding protein.

cose utilization were simultaneously determined have now clearly established that focal physiological cortical activation results in a metabolic uncoupling whereby the increases in blood flow and glucose utilization are not matched by a commensurate increase in oxygen consumption, indicating nonoxidative glucose utilization (7, 44). These *in vivo* observations, which are consistent with the glutamate-induced glycolysis reported here, have led to the notion of activation-induced glycolysis (7). In addition, they have formed the basis for development of the now increasingly popular imaging technique of functional magnetic resonance imaging. This technique is based on the fact that the magnetic-susceptibility properties of hemoglobin vary with its degree of oxygen saturation. Because physiological stimulation results in increased blood flow and glucose utilization without significantly changing oxygen consumption, it follows that hemoglobin present in the venous blood draining the activated focus is less desaturated than surrounding areas, thus yielding a distinct signal detected by magnetic resonance imaging (45, 46). Thus, the model depicted in Fig. 5 can account for the observations obtained in functional brain imaging studies (40–46), which indicate that the mammalian brain normally shifts to glycolysis as a source of energy during brief increases in neuronal activity.

To summarize, focal physiological activation of specific brain areas is accompanied by increases in glucose utilization; since glutamate is released from excitatory synapses when neuronal pathways subserving specific modalities are activated, the stimulation by glutamate of glucose utilization in astrocytes as described here provides a direct mechanism for coupling neuronal activity to glucose utilization in the brain. In addition, these observations also strongly suggest that glucose utilization, as visualized during physiological activation in humans by positron-emission tomography using $^{18}$F-labeled deoxyglucose or in laboratory animals with the 2DG autoradiography technique, may reflect, at least in part, uptake of the tracer into astrocytes. This conclusion does not question the validity of deoxyglucose-based techniques to map neuronal activity; rather it provides a cellular and molecular basis for these *in vivo* imaging procedures.

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