An Evaluation of Erythrocytes as Plasma Glutamate Scavengers for Enhanced Brain-to-Blood Glutamate Efflux

Yin Wang,1 Miroslav Gottlieb,2 and Vivian I. Teichberg1,3

(Accepted October 3, 2003)

Several acute brain pathological conditions are characterized by the presence of excess glutamate in brain interstitial fluid. We have previously shown that decreasing blood glutamate levels increases the driving force for an enhanced brain-to-blood efflux of glutamate. The present study investigated the glutamate pumping ability of glutamate-depleted erythrocytes both in vitro and in vivo to determine whether the latter could potentially be used in a blood exchange procedure for neuroprotection. We have observed that glutamate is taken up in red blood cells only via a passive diffusive process with a diffusion constant of 0.144/h. When glutamate-depleted blood cells resuspended in 6% hetastarch were injected into recipient rats, using a blood exchange protocol, a decrease of blood glutamate was observed but attributed to plasma dilution. These observations are discussed in light of a novel neuroprotective strategy based on blood glutamate scavenging.

KEY WORDS: Glutamate scavenging; brain-to-blood glutamate efflux; erythrocytes; neuroprotection.

INTRODUCTION

Several intractable acute and chronic degenerative conditions of the brain such as stroke (1), head trauma (2), hemorrhagic shock (3), and AIDS dementia (4), are characterized by the presence of excess levels of the excitatory neurotransmitter glutamate (Glu) in the brain interstitial fluid (ISF) and cerebrospinal fluid (CSF). At such levels, Glu can exert neurotoxic properties and kill neurons; therefore neuroprotective strategies are relentlessly being evaluated with the hope of finding ways to neutralize the deleterious effects of Glu and its ensuing neurological deficits.

In this context, we have made the hypothesis that brain neuroprotection could possibly be achieved by accelerating the still little known process of brain-to-blood Glu efflux. Because this efflux takes place against a ISF/CSF-to-blood Glu concentration gradient, our assumption is that the brain-to-blood Glu efflux should be significantly facilitated by a decrease of Glu concentration in blood.

In a previous paper (5), we demonstrated that such a decrease takes place, both in vitro and in vivo, upon activation of the blood resident enzymes Glu-pyruvate transaminase (GPT) and Glu-oxaloacetate transaminase (GOT) with the Glu cosubstrates pyruvate and oxaloacetate. Repeated additions to blood or intravenous administration of pyruvate and oxaloacetate cause a decrease of Glu both in plasma as well as in the blood cell compartment in which most blood Glu resides. The decrease of blood Glu levels causes an increased Glu efflux from brain CSF to blood. This principle of a blood mediated decrease of a ISF/CSF constituent is thus similar to that underlying the action of intravenous asparaginase that is used to deplete plasma and CSF asparagine in the treatment of acute lymphoblastic leukemia (6).

On the above premises, we reasoned that if the Glu-depleted blood cell compartment would be able to
rapidly pump plasma Glu toward the original cell/plasma Glu concentration ratio of ~6, it could provide a novel blood exchange strategy of blood Glu reduction possibly applicable in emergency cases such as hemorrhagic shock and head trauma in which the observed acute increase of ISF/CSF Glu is thought to initiate the neurodegenerative process.

Previous studies carried out with rat and human blood have emphasized the relative impermeability of erythrocytes to extracellular Glu (7–11), but those were carried out in the presence of an unfavorable Glu concentration gradient, that is, on erythrocytes rich in intracellular Glu. It was thus of interest to investigate the Glu transport properties of Glu-depleted blood cells, determine their possible contribution to the reduction of plasma Glu during blood exchange, and evaluate they potential use for neuroprotection.

**EXPERIMENTAL PROCEDURE**

*Materials.* Glu dehydrogenase was from Roche (Mannheim, Germany); Glu-pyruvate transaminase was from Sigma (Rehovot, Israel). All chemicals were purchased from Sigma unless noted otherwise.

*Blood Glutamate Scavenging.* Blood was collected retroorbitally from 200–250 g Sprague-Dawley rats anesthetized intraperitoneally with 40 mg ketamine and 5 mg xylazine/kg body weight. It was incubated at 37°C in the presence of pyruvate, oxalacetate, or a combination of both, 40 mg ketamine and 5 mg xylazine/kg body weight. It was incubated at 37°C with oxaloacetate and pyruvate, added every 10 min, to a final concentration 1 mM. After 40 min, the blood was centrifuged at 4000 rpm/10 min and the plasma withdrawn. The pellet was resuspended to the original blood volume into a 6% hetastarch solution in 0.9% NaCl. Blood exchange into an anesthetized recipient rat was performed by placing a polyethylene cannula (PE 10) in the femoral vein for blood infusion and a polyethylene cannula (PE 10) in the femoral artery for blood withdrawal. Blood was transfused at a rate 0.75 ml/min using a peristaltic pump while arterial blood was withdrawn at the same rate of 0.75 ml/min with another peristaltic pump. Glu levels in the donor rat blood were monitored after blood withdrawal and during the *in vitro* incubation with oxaloacetate and pyruvate. Glu levels in the recipient rat were monitored by removal of 200-μl blood aliquots from the femoral vein or the femoral artery twice before the beginning of the blood exchange, immediately after the end of the exchange, and 15, 30, 60, 90 min postexchange. The total blood exchange times varied between 12–17 min.

*Hemodilution.* Isovolemic haemodilution was carried out over a period of 25 min by substituting each ml of blood that was removed by an equivalent volume of 6% hetastarch in 0.9% NaCl. Glu levels in the recipient were monitored by removal of 200-μl blood aliquots from each milliliter of blood removed. The remaining 900 μl was centrifuged at 4000 rpm/10 min, and the respective volumes of pellet and supernatant were measured and their ratio was defined as the hematocrit.

**RESULTS**

Figure 1 illustrates the effect of repetitive additions of pyruvate together with oxaloacetate on Glu levels in blood and in its separated cellular and plasma compart-

![Fig. 1. Glutamate levels in blood and in its compartments. Dark bars represent untreated blood and its compartments. Open bars represent blood and its compartments incubated for 60 min with 1 mM pyruvate and oxaloacetate added every 15 min. Averages ± SEM are presented. n represents the sample size.](image-url)