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Regulation of blood L-glutamate levels by stress as a possible brain defense mechanism

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ABSTRACT

Isoflurane-anesthetized rats submitted to a closed head injury (CHI) display a significant decrease of their blood glutamate levels. Having demonstrated that a decrease of blood L-glutamate (glutamate) causes an increase of the driving force for a spontaneous brain-to-blood glutamate efflux, and consequently affords brain neuroprotection, we investigated here the possible mechanisms which can affect blood glutamate levels. Reasoning that the spontaneous decrease of blood glutamate levels post CHI could be part of a stress response, we observed that the stress involved in tail artery catheterization under isoflurane anesthesia does not affect blood glutamate levels. Investigating in naïve rats the stress effectors, we found that corticotropin-releasing factor (CRF) significantly decreased blood glutamate levels. Pretreatment with antalarmine (a selective type-1 CRF receptor antagonist) occludes the CRF-mediated decrease in blood glutamate levels. In contrast, the adrenocorticotrophic hormone (ACTH) did not affect blood glutamate levels. Investigating the effectors of the sympathetic/adrenomedullary system, we observed that in naïve rats, adrenaline but not noradrenaline decreased blood glutamate levels. Confirming the role of adrenaline, propranolol pretreatment (a non-selective β-antagonist) prevented the spontaneous decrease of blood glutamate observed post CHI. On the strength of these results, we further observed that isoproterenol (a $\beta_{1/2}$-selective adrenoreceptor agonist) produced a marked sustained decrease in blood glutamate levels. These results suggest that stress induces a decrease of blood glutamate levels partly via the activation of peripheral CRF receptors and the activation of the β-adrenoreceptors. We propose that this newly identified component of the stress response could be a peripherally mediated defense mechanism of the injured brain against the deleterious effects of excess glutamate.

Introduction

Acute brain insults are characterized by the elevation of L-glutamate (glutamate) in brain fluids to toxic levels (Baker et al., 1993; Bullock et al., 1998; Castillo et al., 2002, 1996, 1997; Rose et al., 2002; Spranger et al., 1996; Zauner et al., 1996; Zhang et al., 2001). In human patients after traumatic brain injury and stroke, the increased levels of glutamate in both cerebrospinal fluid (CSF) and blood have been shown to be correlated with the poor neurological outcome (Castillo et al., 1996; 1997) and it is assumed that the long lasting neurological deficits resulting from these pathological events are largely due to the excitotoxic properties of glutamate.

In an attempt to reduce the deleterious effects of excess glutamate after closed head injury (CHI), we have previously demonstrated that decreasing blood glutamate levels in rats with scavengers such as oxaloacetate and pyruvate increases the glutamate concentration gradient between brain interstitial fluid and blood plasma causing thereby an acceleration of the efflux of excess glutamate from brain fluids into blood (Gottlieb et al., 2003). The enhanced brain-to-blood glutamate efflux produces a very significant improvement of the neurological status of rats after closed head injury (Zlotnik et al., 2008; Zlotnik et al., 2007). In the course of the latter studies, while monitoring the changes of blood glutamate levels, we made the unexpected observation of the occurrence of a spontaneous decrease of blood glutamate levels soon after the brain insult, and made the hypothesis that this decrease of blood glutamate levels after brain trauma might be part of a stress response that aims to abate the deleterious effects of brain excess glutamate.

In this paper, we investigated the stress-related mechanisms that could possibly cause naive rats to decrease their blood glutamate

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levels with emphasis on the hypothalamo–pituitary–adrenal pathway and the sympathetic/adrenomedullary system. We present here results suggesting that that blood glutamate levels are indeed under the regulation of specific stress hormones.

Materials and methods

L-Glutamic acid monosodium salt hydrate, beta-nicotinamide adenine dinucleotide (β-NAD), glycine, K_2CO_3 and hydrazine hydrate were purchased from Sigma. Glutamate dehydrogenase (GDH) was purchased from Roche. Corticotropin-releasing factor, adrenocorticotropic hormone, carboxymethylcellulose sodium medium viscosity and antalarmin hydrochloride were purchased from Sigma. Propranolol hydrochloride (Inderal) was from AstraZeneca UK. Adrenaline, noradrenaline and dexamethasone were from TEVA., Israel. Isoproterenol hydrochloride (Isuprel) was form Hospira, Inc., Lake Forest, IL USA.

Animals

The experiments were conducted according to the recommendations of the Declarations of Helsinki and Tokyo and to the Guidelines for the Use of Experimental Animals of the European Community. The experiments were approved by the Animal Care Committee of Ben Gurion University of the Negev. The experiments were carried out on male rats of the Sprague Dawley strain, weighing approximately 200–350 g. They were housed in groups of 4 per cage, under artificial illumination between 06:00 and 18:00 h. Purina Chow and water were available *ad libitum*. Ambient temperature was 22–23 °C. Each experimental group consisted of 6–8 animals. All experiments were performed between 6:00 and 12:00 h.

Animal anesthesia

Spontaneously breathing rats were anesthetized with a mixture of isoflurane (Minrad Inc., USA; initial inspired concentration 2%) in 100% oxygen (1 l/min). Catheterization of the tail vein was carried out with a BD Neoflon 24 g catheter (Becton, Dickinson and Company., USA) for allowing fluid infusion and drug administration. Catheterization of the tail artery was performed to allow blood sampling and determination of blood pressure and heart rate (hemodynamic monitor, Mennen Medical Inc. Horizon XL-267). Blood samples were analyzed for glutamate levels using the fluorometric method of Graham and Aprison (Graham and Aprison, 1966). Glucose levels were measured with Accu-Chek sensor comfort (Roche).

Drugs

Rat corticotropin-releasing factor (CRF also named CRH) (Sigma) was injected intraperitoneally at a dose of 1 μg/100 g. Porcine adrenocorticotropic hormone (ACTH) (Sigma) was injected intraperitoneally at a dose of 25 μg/kg. Noradrenaline (TEVA., Israel) was infused intravenously for 30 min at 2 μg/100 g/min and 1 ml/100 g. Adrenaline (TEVA., Israel) was infused intravenously for 30 min at 1 μg/100 g/min and 1 ml/100 g. Isoproterenol hydrochloride (Isuprel; Hospira, Inc.) was administrated intraperitoneally at a dose of 50 μg/1 kg.

Antalarmine hydrochloride (Sigma) was dissolved in a sterile, lipid soluble fat emulsion (0.5% carboxymethylcellulose sodium medium viscosity (Sigma) pH 5.5) and was administrated intraperitoneally at a dose of 20 mg/kg. One hour before experimentation, rats were injected with antalarmine or equivolume vehicle. Propranolol hydrochloride (Inderal) (AstraZeneca UK) was injected intraperitoneally at a dose of 10 mg/kg 60 min preCHI.

Closed head injury

After infiltration with 0.5% bupivacaine, the scalp was incised and reflected laterally and a cranial impact of 0.5 J was delivered by a silicone-coated rod which protruded from the center of a free-falling plate as previously described (Shapira et al., 1988). The impact point was 1–2 mm lateral to the midline on the skull's convexity. Following closed head injury (CHI), the incision was sutured and rats were laid on their left side and recovered from anesthesia within 1 h.

Determination of whole blood glutamate

Blood aliquots (200 μl) were deproteinized by adding an equal volume of ice-cold 1 M perchloric acid (PCA) and then centrifuged at 10000×*g* for 10 min at 4 °C. The pellet was discarded and supernatant (150 μl) collected, adjusted to pH 7.2 with 2M K_2CO_3 (40 μl) and, if needed, stored at −80 °C for later analysis. Glutamate concentration was measured using the fluorometric method of Graham and Aprison (Graham and Aprison, 1966). A 15 μl aliquot from PCA supernatant was added to 185 μl of a 0.3M glycine, 0.25 M hydrazine hydrate buffer adjusted to pH 8.6 with 1N H_2SO_4 and containing 0.3 U/μl of glutamate dehydrogenase in 0.2 mM NAD^+. After incubation for 0–10 min at room temperature, the fluorescence was measured at 460 nm with excitation at 350 nm. A glutamate standard curve was established with concentrations ranging from 0 to 6 μM. All determinations were done at least in duplicates. The results are expressed as mean ± SEM.

The data were normalized and averaged to prevent the intrinsic variations of basal blood glutamate levels between individual rats (~20%) from masking the effects of the treatment unless very large numbers of animals are studied. With the normalization procedure, each rat serves as its own control and deviations from the basal blood glutamate level can be reliably observed in groups of 6–8 rats.

Determination of blood corticosterone levels

Blood corticosterone concentration was measured by competitive immunoassay direct chemiluminescent technology using ADVIA Centaur XP Immunoassay System of SIEMENS. All determinations were done at least in duplicates. The results are expressed as mean ± SEM.

Statistical analysis

The a priori hypothesis was that the glutamate concentrations in blood samples would differ for treatment groups versus controls and within the treated-groups as a factor of time. Accordingly, the comparison within treated-groups over time was made either with a Student's *t* test (2 points) or with one way analysis of variance (ANOVA; >3 time points) with Bonferroni post hoc testing. Differences were considered as significant when $P<0.05$. The comparisons between different treatment types were assessed using one way analysis of variance (ANOVA) with Bonferroni post hoc testing. The minimal level of significance accepted was $P<0.05$. Data are presented as means ± SEM. Differences were considered as significant when $P<0.05$.

Results

CHI results in a spontaneous decrease of blood glutamate levels

In a previous work (Zlotnik et al., 2007), we made the unexpected observation of the occurrence of a significant spontaneous 20% reduction of blood glutamate levels soon after inflicting a closed head injury to anesthetized rats. In order to confirm this observation, we carried out a new set of experiments and submitted isoflurane-