Impact of methamphetamine on the neuron-astrocyte glutamatergic cross-talk

Ana S. Travassos[1], Teresa Morgadinho[1], Joana Gonçalves[1], Ana P. Silva[1], Carlos Fontes Ribeiro[1] & Frederico C. Pereira[1]

[1]Institute of Pharmacology and Experimental Therapeutics/Biomedical Institute for Research on Light and Image (IBILI), Faculty of Medicine, University of Coimbra

Abstract

Methamphetamine (METH) use could lead to addiction, paranoid schizophrenia-like symptoms, attention and memory deficits, learning problems and compromised decision making. We and others showed that methamphetamine induces an aberrant striatal dopaminergic transmission. Additionally, our group demonstrated that the glutamatergic system is altered following METH exposure. The brain glutamatergic regulation is intimately related to the glutamate-glutamine cycle between neurons and astrocytes. The effect of METH on this neuron-astrocyte cross-talk is not understood. The aim of this work was to assess the impact of a single-high dose of METH on astrocyte-related glutamatergic function in rodents by evaluating the following markers: i) the glutamine/glutamate ratio; ii) glutamine synthetase (GS), an astrocytic enzyme which recycles synaptic glutamate; iii) glial fibrillary acidic protein (GFAP), a structural astrocytic protein.

Adult C57BL/6 mice (3-4 month-old) were sacrificed 4, 24 and 72h following a single-dose regimen of METH (30 $mg.kg^{-1}$ intraperitoneal). Caudate-putamen, frontal cortex and hippocampus were dissected for the analysis of glutamate (GLU), glutamine

(GLN) and glutamine/glutamate ratio, GS and GFAP. Aminoacids were evaluated by HPLC-ED, whereas protein expression levels were assessed by Western blot.

METH treatment evoked an increase in striatal glutamine/glutamate ratio at 72 h, and also a two-fold increase of GFAP and GS expression at this time-point. Frontal cortex exhibited an increase in glutamine/glutamate ratio at 24 h when compared to 4 h and a 3-fold increase in GS expression at 72 h comparing to control. Frontal cortex and hippocampus showed an early 50-60% increase in GFAP expression at 24 h that remained overexpressed at 72 h in frontal cortex. Although hippocampal glutamine/glutamate ratio at 4 h was different from control and 24 h, GS expression remained unchanged throughout the 3 studied time-points. Our results suggest that this METH regimen produced astrogliosis in all tested regions and that astrocytes play a role on glutamatergic neurotransmission disorder.

Key words: methamphetamine, neurotoxicity, reactive gliosis, glutamate-glutamine cycle, GS, GFAP

Introdution

Amphetamines wide abuse have surpassed cocaine and heroin as the most extensively used illicit drugs according to a UN report (www.unodc.org/unodoc/en/global_ilicit_drug_trends.html), leading to an increasing medical and social sequelae. Methamphetamine, a substituted amphetamine, is used to cause euphoria, sense of high work capacity with attentiveness and energy, self-confidence and decreased anxiety (Anglin et al, 2000). However, its cronic abuse can lead to paranoid schizophrenia-like symptoms, attention and memory deficits and compromised decision making, probably related to its aberrant striatal dopaminergic transmission (Cadet et al., 2003). The permanent damage to striatal dopaminergic and

serotonergic nerve terminals (Kita et al.,2003) with depletion of dopamine and serotonin, loss/decrease of tyrosine hydroxylase and tryptophan hydroxylase activity (Hotchkiss and Gibb, 1980; Kita et al., 2003; Pereira et al., 2006), inactivation of dopamine and serotonin transporters (Gibb et al, 1997) have already been studied.

It was recently reported that an increase in extracellular striatal DA levels may cause a secondary corticostriatal release of glutamate via a striato-thalamo-corticostriatal circuit. (Mark et al., 2004, 2007). It was also suggested that increased levels of glutamate could play a role in METH-induced neurotoxicity (Nash and Yamamoto, 1992; Mark et al. 2004, 2007; Caligiuri and Buitenhuys, 2005). For example, pretreatment with MK-801, an N-methyl-D-aspartate (NMDA)-type glutamate receptor antagonist, attenuates METH-induced striatal neurotoxicity (Bowyer et al, 1991). Our group recently showed that METH 30 $mg.kg^{-1}$ induced alterations on NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) glutamate receptor subunits levels in rat caudate-putamen, frontal cortex and hippocampus 24 h post-treatment (Simões et al, 2007, 2008).

The excitatory glutamatergic regulation is intimately related to the glutamate-glutamine cycle between neurons and astrocytes. Glutamate released during synaptic transmission is transported into astrocytes (Danbolt, 2001), where is amidated to glutamine by glutamine synthetase (GS), an enzyme present in astrocytes but absent from neurons (Norenberg and Martinez-Hernandez, 1979; Suárez et al.,2002). Glutamine is released from astrocytes into extracellular space and rapidly taken up into neurons, and catalyzed into glutamate by phosphate-activated glutaminase (PAG) (Kvamme et al, 2000). Finally, glutamate is transported into synaptic vesicles, completing the cycle (Fremeau et al., 2004).

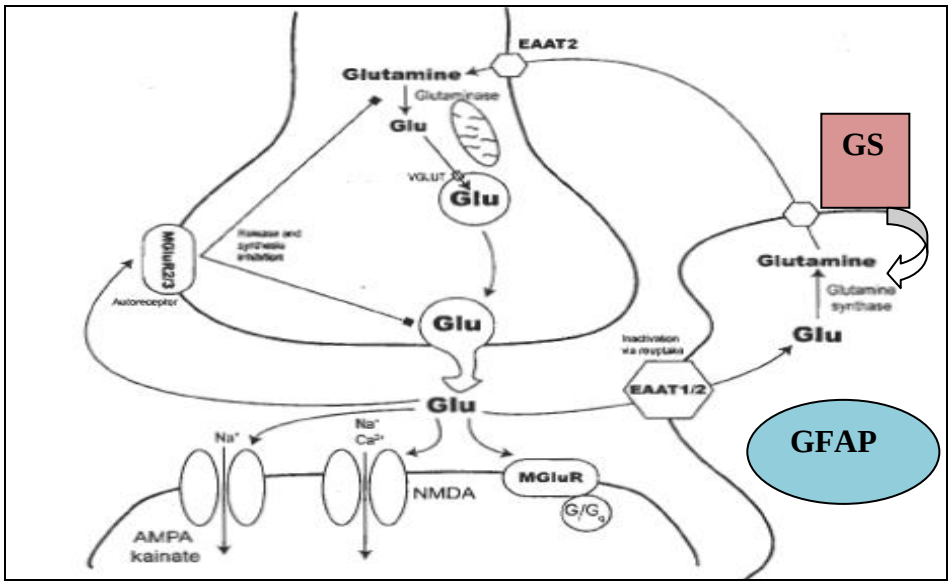

Figure 1- Biochemical mechanisms for glutamate synthesis, release and reuptake. Glu:glutamate; VGLUT: vesicular glutamate transporters; EEAT: excitatory amino acid transporter; mGluR: metabotropic glutamate receptor; NMDA: N-Methyl-D-aspartate/glutamate receptor; AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propinoic acid/glutamate receptor. Adapted from Jentsch JD, Roth RH (2007) Synaptic Transmission: intercellular signaling. Handbook of Contemporary Neuropharmacology

In humans, the use of proton magnetic resonance spectroscopy (MRS) method, seems to provide direct evidence of a neuronal glial glutamate cycle dysfunction with elevated frontal brain glutamate concentration during the cerebral adaptation to methamphetamine abuse (Sailasuta et al., 2009; Abulseoud et al., 2009).

Glutamine/glutamate ratio provides a window into the integrity of neuronal-glial coupling (Ongur et al, 2008). The effect of METH on this neuron-astrocyte cross-talk is not understood. The aim of this work was to assess the impact of a single-high dose of METH on astrocyte-related glutamatergic function in rodents by evaluating the

following markers: i) glutamate (GLU) and glutamine (GLN) total content and glutamine/glutamate ratio; ii) glutamine synthetase (GS), an astrocytic enzyme and iii) glial fibrillary acidic protein (GFAP), a structural astrocytic protein.

Materials and Methods

2.1. Animals

Adult C57BL/6 mices (Charles River Laboratories, Barcelona, Spain), weighing 20-28 g, were housed 4 per cage under controlled environmental conditions (12-h light/dark schedule, at room temperature of 21 ± 1º C, with food and water supplied *ad libitum*). They were subsequently housed individually after injection. All experiments were performed in accordance with European Community guidelines (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs and Chemicals

Methamphetamine.HCl (Sigma Aldrich, Saint Louis, MO, USA). Standards for glutamate and L-glutamine, *o*-phthalaldehyde (OPA solution) and β–mercaptoethanol (MCE) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The other used chemicals *(ultrapure* and *pro analysis* quality) were purchased from Sigma-Aldrich and Merck AG (Darmstadt, Germany).

2.3. Drug Administration and Tissue Sample

Animals were injected intraperitoneally with a single-dose of METH (30 $mg.kg^{-1}$; i.p.).

Control animals were injected with saline solution (0.9%). Animals were sacrificed by decapitation 4, 24 and 72 h post treatment and brains were immediately removed. It was previously demonstrated that METH produced striatal dopaminergic

neurotoxicity at these time-points (Cappon et al, 2000; Pereira et al, 2002; 2006). Caudate-putamen, frontal cortex and hippocampus were dissected on ice, using landmarks from The Mouse Brain in Stereotaxic Coordinates (Paxinos and Franklin, 2004) and stored at -69 ºC until assayed. Left areas were used for protein expression and right areas for glutamate and glutamine analysis.

2.4. Glutamate and glutamine analysis

2.4.1. Sample preparation for the amino acids brain levels

Brain samples were thawed in batches of not more than 8 samples, weighted and homogenized in 0.1 M perchloric acid (150 μL to caudate-putamen and hippocampus and 1500 μL to frontal cortex). The homogenates were then centrifuged (15,000 × g, 3 min) and filtered. The samples were kept at 4º C until HPLC analysis. GLU and GLN were measured as their *o*-Phtalaldehyde (OPA)/β-mercaptoethanol derivatives according to Donzanti and Yamamoto (1988) and Smolders *et* al. (1995). The working OPA/β-mercaptoethanol solution was prepared by adding 2.3 μL of β-mercaptoethanol solution with 1ml of OPA solution.

Ten microliters of the sample were mixed with 2 μL of OPA/β- mercaptoethanol during 2 minutes at room temperature and 2 μL of iodoacetamide solution were further added. Ten microliters of this mixture were injected 2 minutes after.

2.4.2. Chromatographic (*HPLC*) system

The isocratic HPLC method with electrochemical detection used to separate and determine the GLU and GLN levels consisted of a 307 *Gilson* pump, a *Gilson* 231 autosampler, a Spherisorb ODS column (250 × 4.6 mm, 5 μm paticle size), and a 141 *Gilson* amperometric detector with a glassy carbon electrode set at 700 mV versus Ag/AgCl reference electrode. The mobile phase consisted of 0.1 M sodium acetate at

pH = 5.9 containing 10% acetonitrile and 40 mg/L of Na_2EDTA. The flow rate was 1.2 ml/min. Data were analyzed using a *Gilson* Unipoint 5.1 software.

Concentrations of glutamate and glutamine were determined from standard curves generated for each analyte of interest, and values expressed as pmol/10µl/mg tissue.

2.5. GS and GFAP assays

GS and GFAP expression were determined using the Western blot technique. Total tissues were homogenized in lysis buffer (50 mM Tris-HCl -pH 7.4-5% Triton X-100, 4ºC), supplemented with a protease inhibitor misture (1mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT), 5 µg/ml chymostatin, 5 µg/ml leupeptin, 5 µg/ml antiparin, 25 µg/ml Pepstatin A) (Sigma-Aldrich), followed by centrifugation (14 000 rpm, 4º C, 10 min). Total protein concentration was determined with the BCA method (Smith et al., 1985) and homogenates were stored at -69º C until further use. Samples were prepared with sample buffer and then heated at 95º C for 5 min. The concentration of protein needed for visualization of immunopositive bands of GS, GFAP and β-actin was experimentally pre-determined to give a signal that was linear over a large range of detected protein.

2 and 100µg protein for GFAP and GS, respectively, were loaded and separated by electrophoresis on 8% of SDS-PAGE, and transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, Madrid, Spain). Blots were blocked with 5% nonfat dry milk in phosphate buffer saline-PBS for 1 h at room temperature.

Afterwards, the membranes were probed with the primary antibodies - rabbit anti-GFAP polyclonal antibody (1:20 000, 5% milk) or goat anti-GS polyclonal antibody (1:500, 5% milk) (Santa Cruz Biotechnology, USA) - overnight at 4ºC. Then membranes were incubated with an alkaline phosphatase-conjugated secondary

antibody: anti-rabbit or anti-goat (1:20 000; Amersham, GE Healthcare Life Science, USA).

Finally, membranes were visualized using an enhanced chemifluorescence (ECF) detection reagent (Amersham Biosciences, GE Healthcare Europe GmbH, Carnaxide, Portugal) on the Storm 860 Gel and Blot Imaging System (Amersham, GE Healthcare, Buckinghamshire, UK).

To confirm equal protein loading and sample transfer, membranes were reprobed with β-actin. First, to remove the ECF, membranes were washed in methanol 40%, then in water, incubated in a stripping solution (0,2 % mercaptoetanol,10% SDS, 1M Tris base) for 30 min at 50ºC and washed. Next, membranes were blocked as described previously, then incubated with mouse monoclonal anti-β actin (1:10,000; Sigma-Aldrich) overnight at 4°C and afterwards with a secondary anti-mouse (1:2,000; Amersham Biosciences). Membranes were visualized using the same material and equipment.

Using specific antibodies for the proteins in study, we detected prominent gray bands at the molecular weights of 55 kDa for GFAP and 45 kDa for glutamine synthetase. Then, to quantify, membranes background were subtracted, and the gray scale values were averaged and converted to optical density. Densiometric analyses were performed using the Image Quant software. Results were expressed as percentage of control.

2.6. Statistical analysis

Results were expressed as means ± S.E.M. Statistical analysis of differences between methamphetamine-treated group and controls was performed by using one-way analysis of variance ANOVA followed by post-hoc comparison (Fisher´s LSD and Dunnett´s). Significant differences were defined at $p < 0.05$.

Results

Brain regional content of glutamate and glutamine and glutamine/glutamate ratio

Glutamate tissue levels in caudate-putamen, frontal cortex and hippocampus from control animals were 158.4 ± 49.0, 422.6 ± 39.0 and 114.6 ± 20.4 pmol.10μl^{-1}.mg^{-1}, respectively. A single dose of 30 mg/kg of methamphetamine did not change glutamate tissue levels both in caudate-putamen and hippocampus at the three studied time-points (4, 24 and 72 h). However, cortex glutamate content was lower at 24 h comparatively to 4 h (80 vs. 116 % of the control) (fig. 2).

Caudate-putamen, cortical and hippocampal glutamine tissue levels in control animals were 125.9 ± 40.8, 338.8 ± 32.0 and 110.34 ± 29.4 pmol.10ul^{-1}.mg^{-1}, respectively. Methamphetamine evoked only an increment in glutamine levels in hippocampus (152 % of the control) at 4 h comparatively to control and 24 h (fig 2).

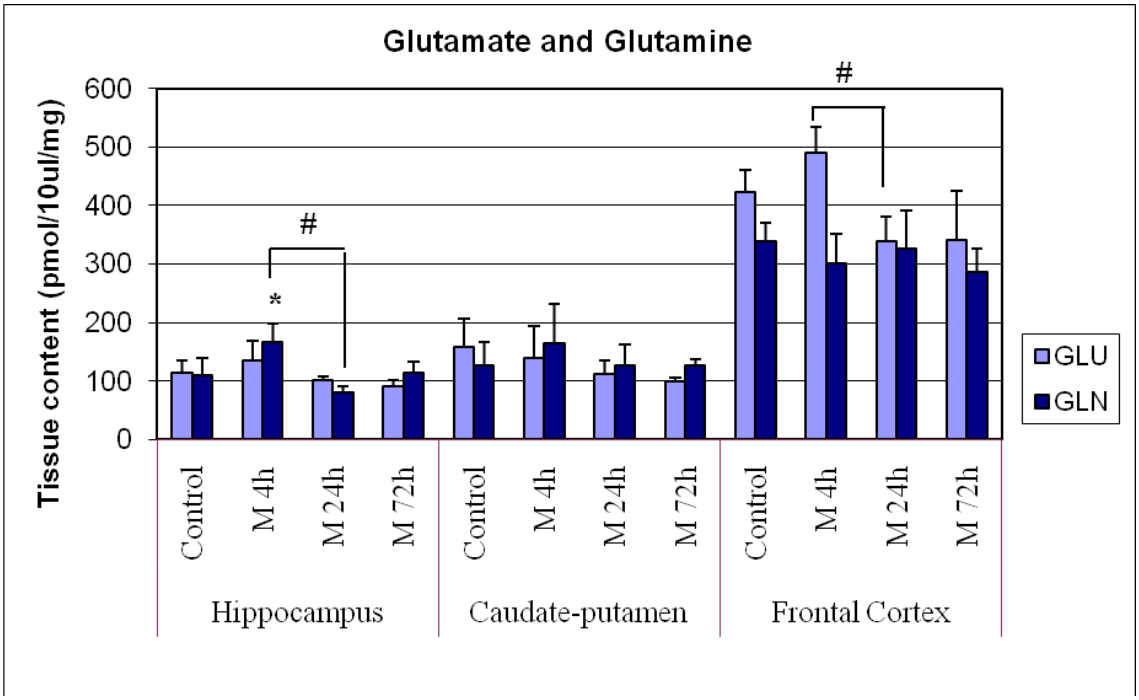

Figure 2- Glutamate (GLU) and glutamine (GLN) tissue content in the three studied areas after a single dose of methamphetamine at 4, 24 and 72h.* P <0.05 significantly different as compared with control; # P < 0.05 significantly different between 4 and 24 h. Data are expressed as mean ±SEM (n= 4-7 animal).

The ratio of glutamine/glutamate was altered in all studied areas: firstly at 4 h in hippocampus (140 % of the control, different from other time-points), secondly at 24 h in frontal cortex (116 % of the control, different from 4 h) and finally at 72 h in caudate-putamen (166 % of the control) (fig. 3).

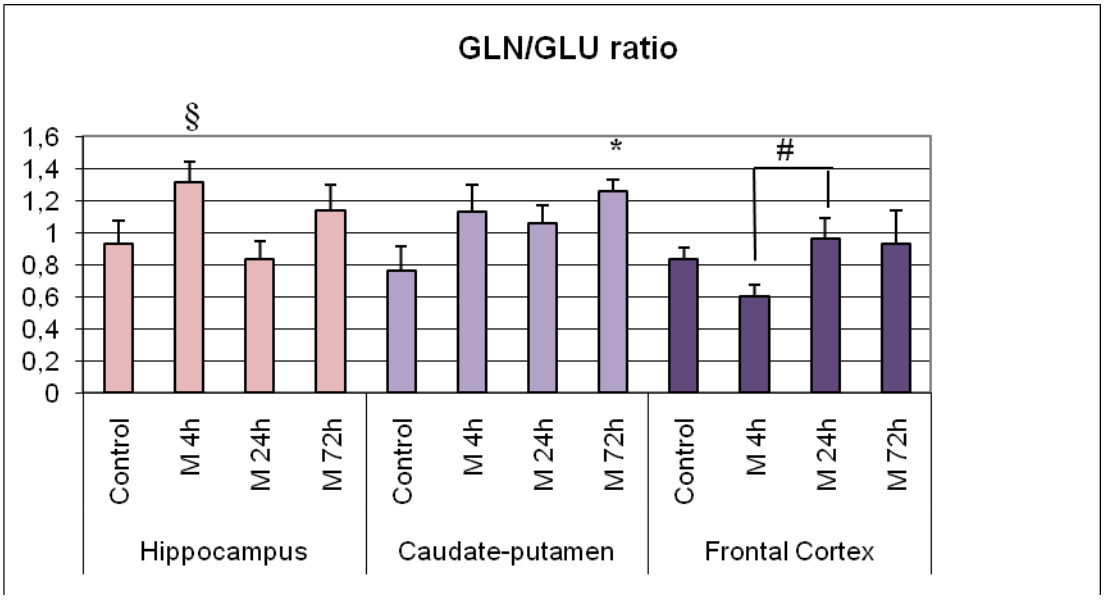

Figure 3- Glutamine/ glutamate ratio after methamphetamine administration in the three studied areas at 4, 24 and 72h. § $P < 0.05$ significantly different from all the other time point. .* $P < 0.05$ significantly different as compared with control. # $P < 0.05$ significantly different between 4 and 24 h. Data are expressed as a ratio and are presented as mean ± SEM (n= 4-7 animal)

Protein expression of GS and GFAP

Methamphetamine evoked an increment in the expression of GS at 72 h in caudate-putamen and frontal cortex (223 and 311% of the corresponding control, respectively). Nevertheless, we did not detect a significant effect on this protein expression in hippocampus in any studied time-point (fig 4).

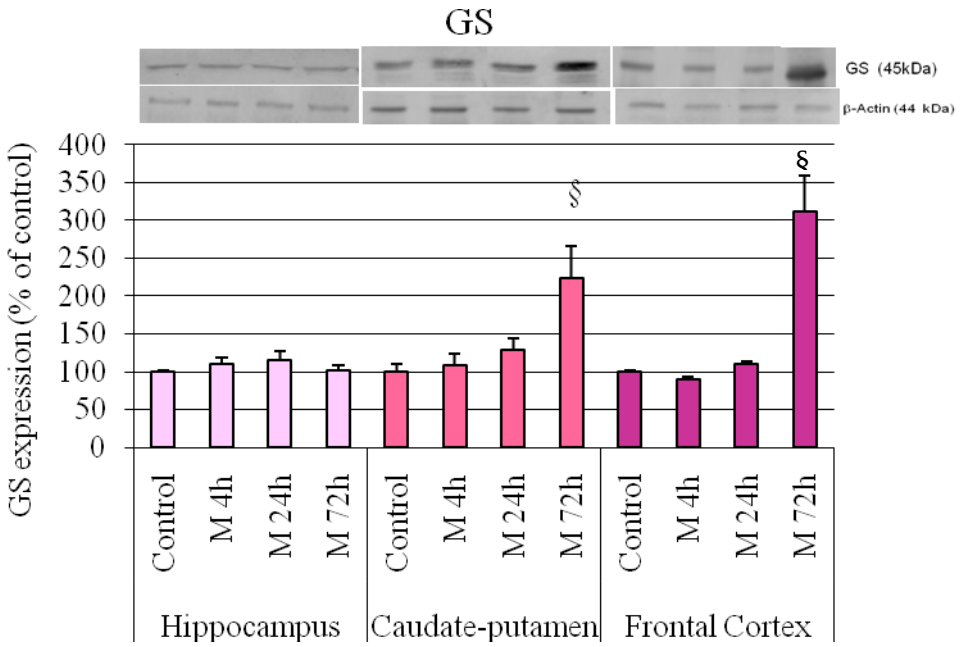

Figure 4- GS expression in the three studied areas after methamphetamine administration at 4, 24 and 72h. § $P < 0.05$ significantly different from all the other time-points. Data are expressed as % of control and are presented as mean ± SEM (n= 4-7 animal).

This drug regimen increased GFAP expression in all studied regions. We found overexpression of this protein at 72 h (213% of control) in caudate-putamen. We detected GFAP over-expression at 24 h in frontal cortex which remained elevated at 72 h (158 and 162% of control, respectively). Finally, methamphetamine evoked an increment in GFAP expression at 24 h (149% of control) in hippocampus (fig 5).

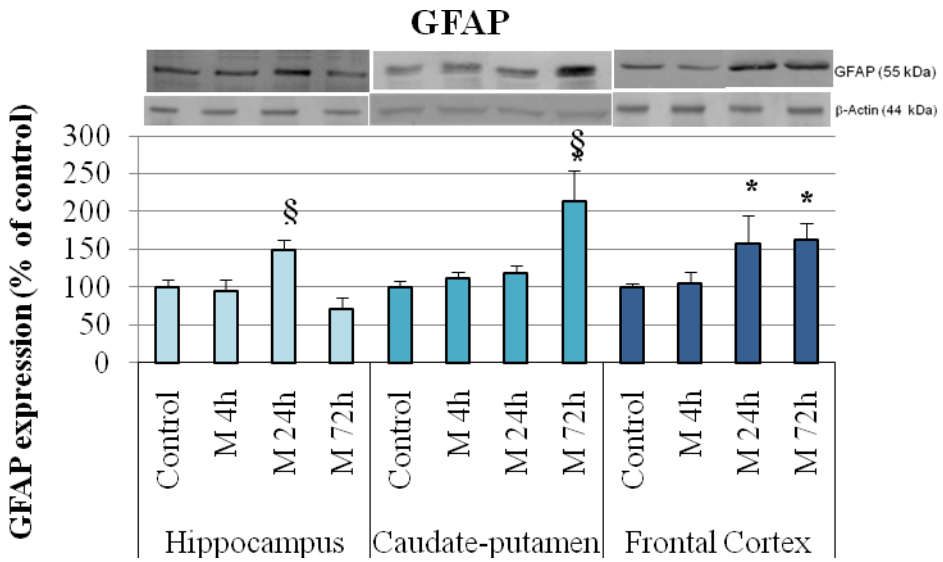

Figure 5- GFAP expression at 4, 24 and 72h in the three studied areas following methamphetamine administration. § $P < 0.05$ significantly different from all the other time point.* $P < 0.05$ significantly different as compared with control. Data are expressed as % of control and presented as mean ± SEM (n= 4-7 animal).

Discussion

The brain glutamate homeostasis plays an important role in pathophysiological processes (Lipton and Rosenberg, 1994; Price, 1999).

The present study aimed to characterize the time-course of the impact of a single injection of a high dose of methamphetamine (30 $mg.kg^{-1}$) on the glutamate-glutamine cycle in different brain regions. This acute dose mimics the common human overdose scenario, when a new abuser will take the same high dose that a tolerant abuser is capable of, but which will be lethal to the first one (Simões et al., 2007).

Methamphetamine (METH) is a powerful psychostimulant that increases extracellular glutamate (GLU) levels in the mammalian brain (see introduction, Stephans and Yamamoto, 1994). Glutamate is an ubiquitous aminoacid that is required

by all cells for its role in protein synthesis and intermediary metabolism. Its levels are extremely high in CNS approaching 5-10 mmol/kg (for review, Danbolt, 1994). Many glutamate receptors are activated by low extracellular micromolar concentrations (1-10 μM) (Sheldon and Robinson, 2007). Extracellular concentration of this neurotransmitter must be maintained at relatively low levels to ensure an appropriate signal-to-noise ratio and to prevent excessive activation of glutamate receptors that can result in cell injury or death, a phenomenon known as excitotoxicity (Hansson and Ronnback 2005; Sheldon and Robinson, 2007).

It is generally accepted that the neurotransmitter pool of glutamate is maintained by the glutamate-glutamine cycle (Rodrigo and Felipo, 2007), which is supported by a cross-talk between neurons and astrocytes. Besides having a major role in metabolic support (glucose–lactate shuttle) (Benarroch, 2005), osmolarity, volume and pH regulation, this glial cells are bidirectional communication partners in the CNS, receiving signals from neighboring neurons, as demonstrated by the expression of same receptors, and responding with release of neuroactive substances (Hansson et al., 1995, Araque et al, 1999, Bezzi, 2001). Astrocytes exert a key control of the glutamate homeostasis, clearing a great percentage (80%) of glutamate released following neuronal activity, while the rest is accumulated into postsynaptic neurons (Verkhratsky and Kirchhoff, 2007). Excitotoxic neuronal damages resulting from excessive glutamate levels are frequently associated with impaired handling of extracellular glutamate by astrocytes (Seifert et al., 2006).

In our study, we measured total content of glutamate and glutamine after methamphetamine administration. Glutamate content of brain homogenates mainly reflect a neuronal pool, since glia are relatively poor in this neurotransmitter (Waagepetersen et al., 2003). We did not find significant changes in glutamate neither

in hippocampus nor in caudate-putamen, but we found a slight decrease in frontal cortex. Considering that brain glutamate is essentially intracellular, its levels might not reflect glutamatergic synaptic activity (Ongur et al., 2008). Relatively to results from the first two areas, glutamate levels might be not only replenished by the neuron-astrocyte cycle but also by tricarboxylic acid cycle, warranting glutamate homeostasis. Glutamate reduction found in frontal cortex (20% of the control) might be related to its recruitment to oxidation for energy, via the tricarboxylic acid (TCA) cycle, incorporation into proteins or synthesis of GABA (γ-aminobutyric) (McKenna, 2007) with a failure in reposition of normal levels. Alternatively, glutaminase could be dysfunctional contributing to this hampered GLU homeostasis in frontal cortex. Finally glutamate could be used as a precursor for GABA synthesis

Simultaneous glutamine measurements and glutamate/glutamine ratio might better capture changes in neuron-astrocyte cross-talk. Glutamine is the molecule that couples ammonia metabolism to the synthesis of neurotransmitter aminoacids glutamate and γ-aminobutyric acid (GABA) (Albrecht et al., 2007). The majority of CNS glutamine is synthesized endogenously by glutamine synthetase (GS) in astrocytes (see introduction). Tissue glutamine levels reflects predominant intracellular content (Erecinska and Silver, 1990), of which 80% is estimated to be in glia (Ottersen et al.,1992). Glutamine concentration in astrocytes also reflects the amount of glutamate uptake (Bak, 2006).

In our study, we found an increased (50%) glutamine total content levels in hippocampus at 4 h, but no statistically significant change in caudate-putamen and frontal cortex. Instead, we noticed an elevation of GLN/GLU ratios in all the studied regions in methamphetamine injected mice, starting in hippocampus, followed by frontal cortex and finally in caudate-putamen. Since that in normal conditions glutamate

provides an overwhelming majority (80-90%) of the substrate pool for glutamine synthesis (Kanamori et al., 2002), these last results are potencially due to enhanced conversion of glutamate to glutamine by glutamine synthetase (GS) in astrocytes.

We also studied a functional and a structural astrocytic proteins [glutamine synthetase (GS) and GFAP] to better evaluate astrocytes behaviour in the presence of methamphetamine.

Glutamine synthetase (GS) expression and activity is correlated with extracellular glutamate concentration (Derouiche and Frotscher, 1991; Fonseca et al., 2005). For example, it is known that cultured astrocytes respond to glutamate with increased GS activity. Moreover, the distribution of both glial glutamate receptors and transporters parallels the GS location suggesting a functional coupling between glutamate uptake and degradation (Suarez et al, 2002). It also plays a central role in the maintenance of the synaptic pool of glutamate (Laake et al., 1995).

Methamphetamine induced a 2 to 3 times increase in GS expression in caudate-putamen and frontal cortex, respectively, both at 72 h. The results in the first two areas are in harmony with the increased glutamine/glutamate ratio. Because glutamate can be excitotoxic, elevation of GS expression might be part of an endogenous mechanism for neuroprotection. Regarding hippocampus, although we did not see any changes in GS expression, the increment in GLN suggest that the enzyme activity was increased by METH.

Reactive gliosis or astroglia activation - an early response to brain damage in many CNS pathologies, such as stroke, trauma, neurodegenerative disease such as Parkinson´s or Alzheimer´s disease, or other conditions like epilepsy, schizophrenia and depression (Miyatake et al.2005, Halassa et al, 2006) - usually courses with hypertrophy

of astrocytes processes and the up-regulation of intermediate filament proteins, in particular GFAP (O'Callaghan and Miller, 1993; Pennypacker et al., 2000). This protein is perhaps the best known hallmark of reactive astrocytes (Raivich et al., 1999; Pekny and Nilsson, 2005). The reactive astrocytes are not only important for rescuing neurons in the injured area but also for clearing up excessive glutamate at the synaptic level. Astroglia protection against glutamate excitotoxicity became obvious in *in vitro* experiments: withdrawal of astrocytes from neuronal cultures invariably produced a significant increase in neuronal death triggered by glutamate administration (Danbolt, 2001). Nevertheless, besides playing a neuroprotective role (limiting the area of damage by scar formation) the activated astrocytes may also have a neurotoxic role (with detrimental signals that contribute to the disorder), depending on the character and extent of the damage as well as the timepoint (Miyatake et al.2005, Benarroch, 2005, Giaume et al., 2007).

In this study, we observed that acute administration of METH induced a GFAP overexpression in all studied areas, corresponding to a significant reactivity of the astrocytes, confirming that the used dose was neurotoxic. Our results are consistent with METH-inducing astroglia activation in the cortex, caudate-putamen and hippocampus of rodents (Pubill et al., 2003).

In summary, the present study showed that a single injection of a high dose of methamphetamine (30 $mg.kg^{-1}$) induced overexpression of GFAP and increased ratio of glutamine/glutamate in all studied regions. The balance in the glutamate-glutamine cycle seems to be disrupted in all tested areas in different periods of time. Analysing results by region, we noticed early changes in hippocampus with increased ratio of glutamine/glutamate due to elevated tissue levels of glutamine, followed by overexpression of GFAP at 24 hours. Frontal cortex revealed elevated ratio

glutamine/glutamate due to reduced levels of glutamate at 24 hours. GFAP overexpression was noticed at same time course, maintained at 72 hours, along with overexpression of glutamine synthetase in the last time-point. Caudate-putamen showed changes only at 72 h, with increased ratio of glutamine/glutamate, as well as overexpression of the two proteins analysed.

	Hippocampus	**Frontal Cortex**	**Caudate-putamen**
GLU e GLN	4h (+ GLN)	24h (< GLU)	NS
GLN/GLU	4h (+ GLN)	24h (+)	72h (+)
GFAP	24h (+)	24 e 72h (+)	72h (+)
GS	NS	72h (+)	72h (+)

Table 1- The time-course of the effects of a single dose of methamphetamine on tissue content of glutamate and glutamine, glutamine/glutamate ratio and GFAP and GS expression in hippocampus, frontal cortex and caudate-putamen.

Conclusion

Brain pathology is, to a great extent, a pathology of glia, which, when failing to function properly, determines the degree of neuronal death, the outcome and the scale of neurological deficit. Glial cells are central in providing for glutamate homeostasis (Giaume et al., 2007).

The elevated glutamine/glutamate ratio seems to reflect glutamatergic overactivity and/or defective neuronal-glia coupling in methamphetamine use. Consistently, key functional and structural astrocytic molecules involved in enzymatic activity and cytoskeletal integrity are abnormally expressed, suggesting that astrocytes play a role on the glutamatergic dysfunction triggered by METH.

Abnormalities in glutamatergic neurotransmission glial-related may represent targets for novel therapeutic interventions on METH abuse.

References

Abulseoud O, Sailasuta N, Hernandez M, Ross BD (2009) Impact of depression on cerebral and cognitive function in abstinent methamphetamine users. Proc Int Soc Magn Reson Med 17:210

Albrecht J, Sonnewald U, Waagepetersen HS, Schousboe A (2007) Glutamine in the central nervous system: function and dysfunction. Frontiers in Bioscience 12: 332-343

Anglin MD, Burke C, Perrochet B, Stamper E, Dawud-Noursi S (2000) History of the methamphetamine problem. J Psychoactive Drugs 32:137-141

Araque A, Parpura V, Sanzgiri RP, Haydon PG (1999) Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 22:208-215

Bak L, Schousboe A, Waagepetersen H (2006) The glutamate/GABA- glutamine cycle: aspects of transport, neurotransmitter homeostasis and ammonia transfer. Journal of Neurochemistry 98: 641-653

Benarroch E, (2005). Neuron-astrocyte Interactions: Partnership for normal function and disease in the central nervous system. Mayo Clinic Proc. 80(10): 1326-1338

Bezzi P, Volterra A (2001) A neuron- glia signalling network in the active brain. Current Opinion in Neurobiology 11:387-394

Bowyer JF, Scallet AC, Holson RR, Lipe GW, Slikker W Jr, Ali SF (1991) Interactions of MK-801 with glutamate-, glutamine- and methamphetamine-evoked release of [3H]dopamine from striatal slices.J Pharmacol Exp Ther. 257(1):262-70.

Cadet JL, Jayanthi S, Deng X (2003) Speed kills: cellular and molecular bases of methamphetamine-induced nerve terminal degeneration and neuronal apoptosis. FASEB J 17:1775-1788

Caligiuri MP, Buitenhuys C (2005) Do preclinical findings of Methamphetamine-induced motor abnormalities translate to an observable clinical phenotype? Neuropsychopharmacology 30:2125-2134

Cappon GD, Pu C, Vorhees CV (2000). Time-course of methamphetamine-induced neurotoxicity in rat caudate-putamen after a single-dose treatment. Brain Res 863:106-111

Danbolt NC (1994) The high affinity uptake system for excitatory amino acids in the brain.Prog Neurobiol 44(4):377-96.

Danbolt N (2001) Glutamate uptake. Prog Neurobiol 65: 1-105

Derouiche A, Frotscher M (1991) Astroglial processes around identified glutamatergic synapses contain glutamine synthetase: evidence for transmitter degradation. Brain Res 552:346-350

Donzanti BA, Yamamoto BK (1988) An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. Life Sci 43(11):913-22

Erecinska M, Silver I (1990) Metabolism and role of glutamate in mammalian brain. Prog Neurobiolo 35: 245-296

Fonseca LL, Monteiro MA, Alves PM, Carrondo MJ, Santos H (2005) Cultures of rat astrocytes challenged with a steady supply of glutamate: new model to study flux distribution in the glutamate-glutamine cycle. Glia 51: 286-296

Fremeau RT Jr, Voglmaier S, Seal RP, Edwards RH (2004) VGLUTs define subsets of excitatory neurons and suggest novel roles for glutamate.Trends Neurosci 27(2):98-103.

Giaume C, Kirchhoff F, Matute C, Reichenbach A, Verkhratsky A (2007) Glia: the fulcrum of brain diseases. Cell death and Differentiation 14:1324-1335

Gibb W, Johnson M, Elayan I, Lim H, Matsuda L, Hanson G (1997) Neurotoxicity of amphethamines and their metabolites. NIDA Res.Monogr 173:128-145

Halassa M, Fellin T, Haydon P (2006) The tripartite synapse: roles for gliotransmission in health and disease. Trends in Molecular Medicine vol 13,No.2: 54-63

Hansson E, Ronnback L (1995)Astrocytes in glutamate neurotransmission. FASEB Journal 9: 343-350

Hotchkiss AJ, Gibb JW (1980) Long term effects of multiple doses of methamphetamine on tryptophan hydroxylase and tyrosine hydroxylase activity in the rat brain. J Pharmacol Exp Ther 214:257-262

Jentsch JD, Roth RH (2007) Synaptic Transmission: intercellular signaling. Handbook of Contemporary Neuropharmacology Chapter 2:39-58

Kanamori K, Ross B, Kondrat R (2002) Glial uptake of neurotransmitter glutamate from the extracellular fluid studied in vivo by microdialysis and (13)C NMR. J Neurochem 83: 682-695

Kita T, Wagner GC, Nakashima T (2003) Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption. J Pharmacol Sci 92:178-195

Kvamme E, Roberg B, Torgner IA (2000) Phosphate-activated glutaminase and mitochondrial glutamine transport in the brain. Neurochem Res 25:1407-1419

Laake JH, Slyngstad TA, Haug FM, Ottersen OP (1995) Glutamine from glial cells is essential for the maintenance of the nerve terminal pool of glutamate: immunogold evidence from hippocampal slice cultures. J Neurochem 65: 871-881

Lipton SA, Rosenberg PA (1994) Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med 330: 613-622

Norenberg M, Martinez-Hernandez A (1979) Fine structural localization of glutamine synthetase in astrocytes of rat brain. *Brain Res*.161: 303-310

Mark KA, Soghomonian JJ, Yamamoto BK (2004) High dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. J Neurosci 24:11449-11456

Mark KA, Quinton MS, Russek SJ, Yamamoto BK (2007) Dynamic Changes in vesicular glutamate transporter 1 function and expression related to methamphetamine-induced glutamate release. The Journal of Neuroscience 27(25):6823-6831

Mckenna M (2007) The glutamate-glutamine cycle is not stoichiometric: fates of glutamate in brain. Journal of Neurosciense Research 85: 3347-3358

Miyatake M, Narita M, Shibasaki M, Nakamura A, Suzuki T (2005) Glutamatergic neurotransmission and protein kinase C play a role in neuron-glia communication during the development of methamphetamine- induced psychological dependence. European Journal of Neuroscience 22:1476-1488

Nash JF, Yamamoto BK (1992) Methamphetamine neurotoxicity and striatal glutamate release: comparison to 3,4-methylenedioxymethamphetamine. Brain Res 581:237-243

O'Callaghan JP, Miller DB (1993) Quantification of reactive gliosis as an approach to neurotoxicity assessment. NIDA Res Monogr 136:188-212

Ongur D, Jensen JE, Prescot AP, Stork C, Lundy M, Cohen BM, Renshaw PF (2008) Abnormal glutamatergic neurotransmission and neuronal-glial interaction in acute mania. Biol Psychiatry xx:xxx 1-9

Ottersen O, Zhang N, Walberg (1992) Metabolic compartmentation of glutamate and glutamine: morphological evidence obtained by quantitative immunocytochemistry in rat cerebellum. Neuroscience 46: 519-534

Paxinos G, Franklin KBJ (2004) The mouse brain in stereotaxic coordinates, 2th edition. Academic Press Limited, London, UK

Pekny M, Nilsson M (2005) Astrocyte activation and reactive gliosis. Glia 50:427-434

Pennypacker KR, Kassed CA, Eidizadeh S, O'Callaghan JP (2000) Brain injury: prolonged induction of transcription factors. Acta Neurobiol Exp (Wars) 60(4):515-30.

Pereira FC, Imam SZ, Gough B, Newport GD, Ribeiro CF, Slikker W, Macedo TR, Ali SF (2002) Acute changes in dopamine release and turnover in rat caudate nucleus following a single dose of methamphetamine. J Neural Transm 109:1151-1158

Pereira FC Lourenço ES, Borges F, Morgadinho T, Ribeiro CF, Macedo TR, Ali SF (2006) Single or multiple injections of methamphetamine increased dopamine turnover but did not decrease tyrosine hydroxylase levels or cleave caspase-3 in caudate-putamen.Synapse 60(3):185-93.

Price DL (1999) New order from neurological disorders. Nature 399:A3-A5

Pubill D, Canudas AM, Pallas M, Camins A, Camarasa J, Escubedo E (2003) Different glial response to methamphetamine- and methylenedioxymethamphetamine-induced neurotoxicity. Naunyn Schmiedebergs Arch Pharmacol 367:490-499

Raivich G, Bohatschek M, Kloss C, Werner A, Jones L, Kreutzberg G (1999) Neuroglial activation repertoire in the injured brain: graded response, molecular mechanisms and cues to physiological function. Brain Res. Rev. 30: 77-105

Sailasuta N, Abulseoud O, Harris K and Ross B (2009) Glial dysfunction in abstinent methamphetamine abusers. Journal of Cerebral Blood Flow & Metabolism ([Epub ahead of print]) 1–11

Seifert G, Schilling K, Steinhauser C (2006) Astrocyte dysfunction in neurological disorders: a molecular perspective. Nat Rev Neurosci 7:194-206

Sheldon AL, Robinson MB (2007) The role of glutamate transporters in neurodegenerative diseases and potencial opportunities for intervention. *Neurochemistry International* 51: 333-355

Simões P, Silva A, Pereira F, Marques E, Grade S, Milhazes N, Borges F, Ribeiro C and Macedo T (2007) Methamphetamine induces alteration on hippocampal NMDA and AMPA receptor subunit levels and impairs spatial working memory. *Neuroscience* 150: 433–441

Simões PF, Silva AP, Pereira FC, Marques E, Milhazes N, Borges F, Ribeiro CF, Macedo TR (2008) Methamphetamine changes NMDA and AMPA glutamate receptor subunit levels in the rat striatum and frontal cortex. Ann N Y Acad Sci 1139:232-41.

Smith AJ, Cawston TE, Hazleman BL(1985) A rapid and reproducible method for the analysis of immune complexes using affinity chromatography and Western blotting.J Immunol Methods 84(1-2):125-34

Smolders I, Sarre S, Michotte Y, Ebinger G (1995)The analysis of excitatory, inhibitory and other amino acids in rat brain microdialysates using microbore liquid chromatography.J Neurosci Methods 57(1):47-53

Stephans SE,Yamamoto BK (1994) Methamphetamine-induced neurotoxicity: roles for glutamate and dopamine efflux. Synapse 17:203-209

Suárez I, Bodega G, Fernández B (2002) Glutamine synthetase in brain: effect of ammonia. Neurochemistry International 41:123-142

Verkhratsky A, Kirchhoff F. (2007) Glutamate-mediated neuronal-glial transmission. J Anat 210: 651-660

Waagepetersen HS, Sonnewald U, Schousboe A (2003) Compartmentation of glutamine, glutamate, and GABA metabolism in neurons and astrocytes: functional implications. Neuroscientist 9(5):398-403

.