Participation of mTOR in the transport of aminoacids involved in glutathione (GSH) synthesis in mouse brain.

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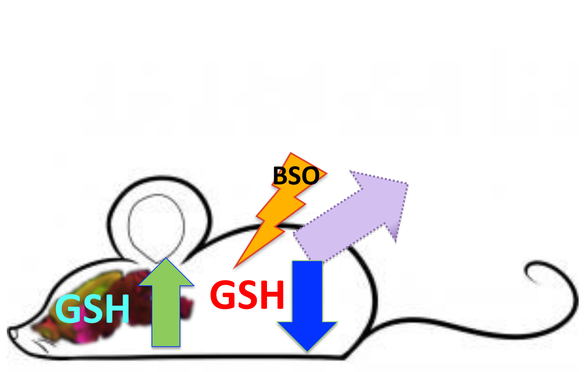
Introduction

Glutathione (GSH) is the most important intracellular antioxidant system. It plays a primordial role in the protection of cells against oxidative stress, mainly in the central nervous system (CNS).

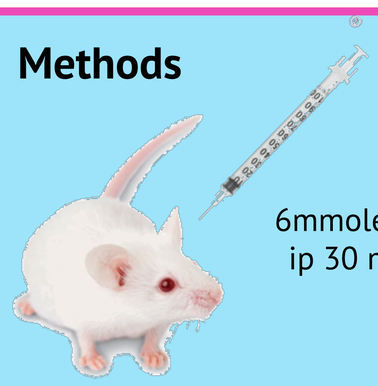
The synthesis of GSH is limited by the availability of cysteine. Cysteine is incorporated into the brain through the blood brain barrier (BBB), and through neurons and astrocytes, where specific transporters like LAT1, xCT and EAAC1 are expressed.

In the mouse striatum the nerve growth factor (NGF) activates an antioxidant response via its receptor TrkA and downstream PKB/AKT through PI3K. The activation of this pathway is associated with the increased transcription of *xct, lat1, eaac1*. On the other hand, TOR (target of rapamycin) a serine/threonine kinase modulates protein synthesis in response to growth factors. Moreover, PKB/AKT indirectly activates mTOR by inhibiting TSC2.

We have evidence suggesting that the systemic oxidative stress is associated with an increase of plasma NGF levels. Thus, we plan to investigate the participation of mTOR in the NGF modulation of cysteine/cysteine transport in the mouse brain.



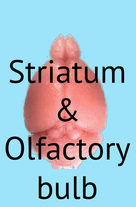
Methods



Ratones BALB/c

n=5

6mmoles/kg BSO
ip 30 min & 2h

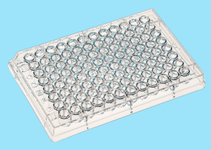


Striatum & Olfactory bulb

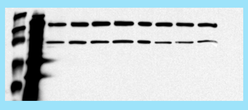
- ✓NGF
- ✓mTOR
- ✓S6K
- ✓xCT
- ✓EAAC1
- ✓LAT1

Protein

Citoplasms & membranes



Bradford



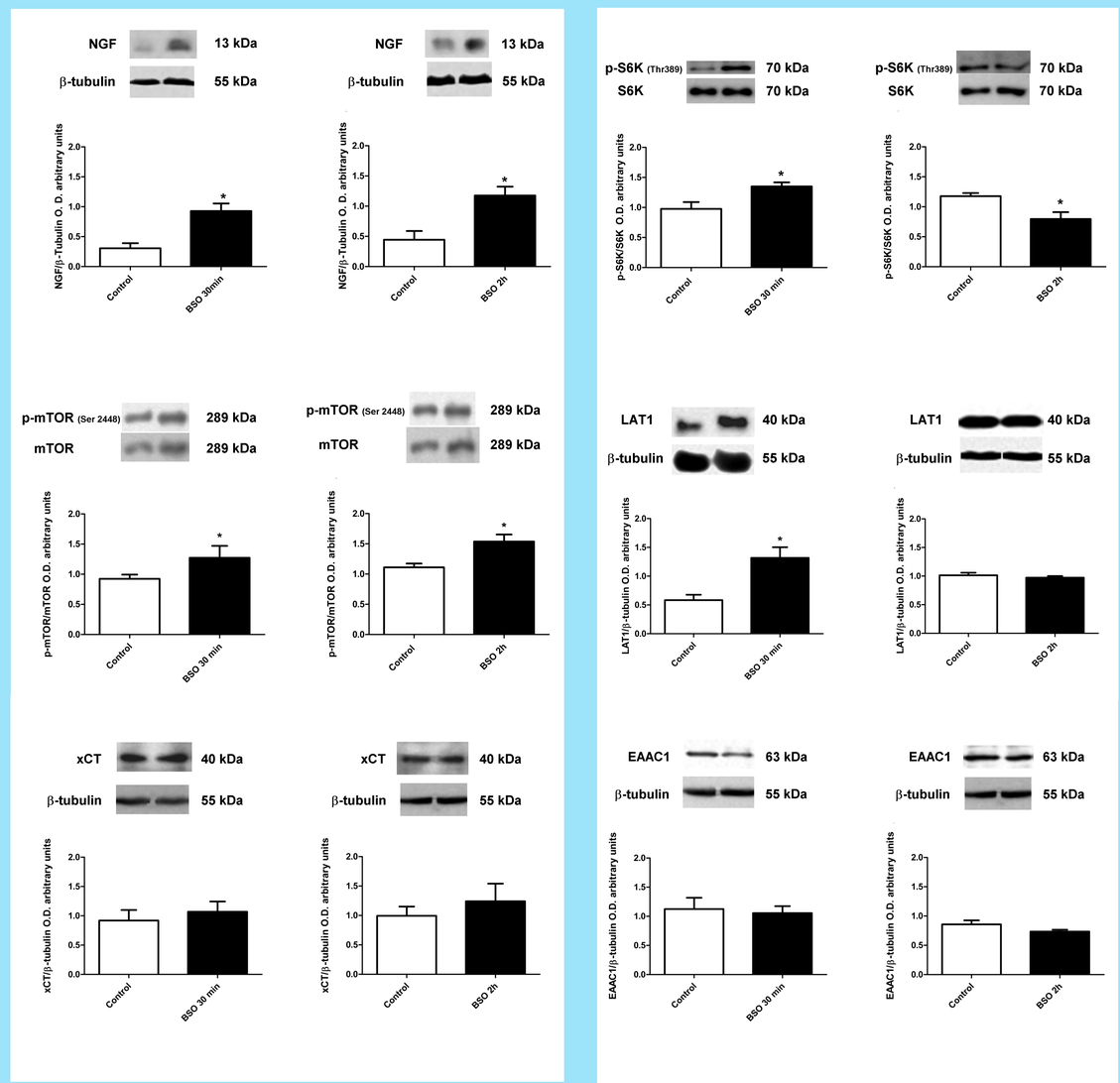
Western blot

ImageJ
Image Processing and Analysis in Java

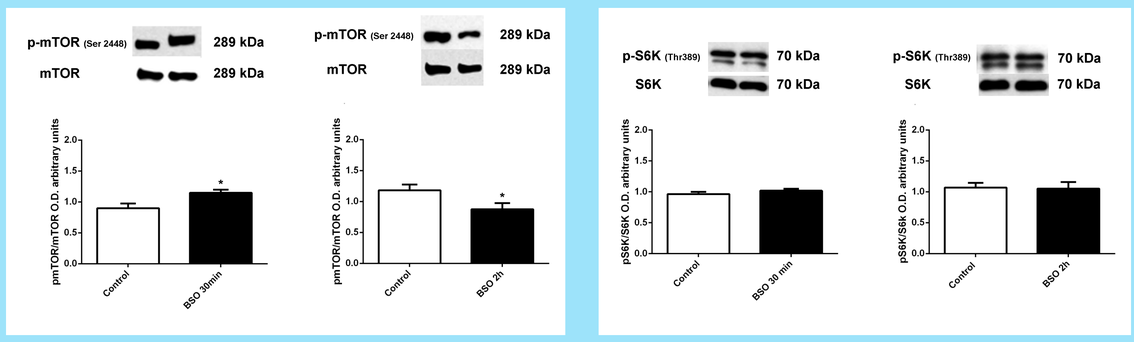
Densitometry

Results

S6K and mTOR phosphorylation and NGF, LAT1, xCT1, and EAAC1 expression levels were evaluated in the mouse striatum and olfactory bulb upon BSO treatment.



S6K and mTOR phosphorylation as well as NGF and LAT1 protein expression levels were found up-regulated in the mice striata at 30 min after BSO injection, while at 2h post injection, only NGF and mTOR, but not S6K or LAT1, remained up-regulated. No changes in xCT1 and EAAC1 protein expression were observed at 30 min and 2h time points.



mTOR phosphorylation levels were found up-regulated in the olfactory bulb of the mice at 30 min after BSO injection, while at 2h post injection there was a down-regulation. The S6K levels did not change with the BSO treatments.

Conclusions

The results show that i.p. BSO administration activates the NGF/mTOR pathway as well as LAT1 transporter in murine striata at 30 min of treatment. On the other hand, this activation seems not to be persistent, because no changes in protein levels of xCT, EAAC1 and LAT1 transporters could be observed at 2h post treatment.

In olfactory bulb, BSO seems to activate mTOR at 30 min and downregulate it at 2h of treatment, while the levels of S6K remain unchanged.

Our results suggest that the response to BSO is region specific.

References

1. Dringen, R. 2000. *Progress in Neurobiology* 62:649-671.
2. Valdovinos-Flores, C. y Gonsebatt, ME. 2012. Neurochemistry International 61:405-414.
3. Limón-Pacheco, J.H., Hernández, N.A., Fanjul-Moles, M.L., Gonsebatt, ME.2007. *Free Radic. Biol. Med.*43, 1335-1347.
4. Kim y Guan. 2012.The Annual Review of Biochemistry. 80:1001-32.
5. Sofroniew, M.V., Howe,C.L. y Mobley, W.C. 2001. Annual Review of Neuroscience 24: 1217-1281.

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