Both aging and chronic fluoxetine increase S100B content in the mouse hippocampus

Mustafa Akhisaroglu, Radmila Manev, Ezgi Akhisaroglu, Tolga Uz and Hari Manev[CA]

The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, 1601 West Taylor Street, MC912, Chicago, IL 60612, USA

[CA]Corresponding Author: HManev@psych.uic.edu

Received 1 April 2003; accepted 21 May 2003

DOI: 10.1097/01.wnr.0000082126.86681.4c

S100B is a cytokine with neurotophic and neurite-extending activity that has been implicated in the mechanism of action of antidepressants and in the pathobiology of aging associated disorders such as Alzheimer's disease. The antidepressant fluoxetine increases hippocampal S100B content in young adult rats. In humans, brain levels of S100B mRNA and protein increase with advancing age. We assayed hippocampal S100B protein content in young (2 month) and old (24 month) mice, and in old mice treated for 2 weeks with fluoxetine. Using quantitative Western immunoblotting and an immunoassay kit we found higher S100B content in the hippocampus of old mice. Fluoxetine treatment of old mice further increased hippocampal S100B, suggesting that aging does not interfere with fluoxetine's action on hippocampal S100B. *NeuroReport* 14:1471–1473 © 2003 Lippincott Williams & Wilkins.

Key words: Aging; Alzheimer; Antidepressant; Fluoxetine; Hippocampus; Mouse; Neurotrophic; S100B; Serotonin

INTRODUCTION

S100B is a member of the S100-calmodulin-troponin superfamily of proteins and is abundantly expressed by CNS glia, and, to a much lower degree, by some populations of neurons [1,2]. S100B is secreted from cells and is believed to act as a cytokine that may have intercellular signaling functions at nanomolar concentrations. S100B, like other S100 proteins, is susceptible to dimerization and can form disulfide cross-linked homodimers in the presence of calcium and lipids. When released, S100B can act both on neurons and on glia [3,4]. For instance, S100B was shown to be capable of promoting neuronal survival and neurite outgrowth in specific neuronal subpopulations; it also enhances neuronal survival during development and after injury, and stimulates astrocyte proliferation *in vitro* [5–9]. It has been speculated that S100B might participate in the pathobiology of aging-associated diseases such as Alzheimer's disease [10] and possibly in the antidepressant actions of pharmacological treatments [11,12].

In the brain, the expression and release of S100B appear to be regulated by the neurotransmitter serotonin (5-HT) and can be affected by antidepressants (e.g. fluoxetine, a selective 5-HT reuptake inhibitor). Thus, the response of astrocytes to pharmacological alterations of 5-HT content has been studied by immunocytochemistry for S100B [13]. In these studies, stereological analysis of the hippocampus revealed a direct relationship between the expression of S100B and the levels of 5-HT. Hence, when 5-HT content was reduced by treating rats with para-chlorophenylalanine (an inhibitor of 5-HT biosynthesis), S100B immunoreactivity was also reduced. An increase in S100B immunoreactivity was observed in cases where 5-HT levels were increased by fluoxetine administration [13]. Using quantitative Western immunoblotting, we recently observed a stimulatory effect of chronic (21 day) fluoxetine administration on hippocampal S100B content [14]. We proposed that alterations of S100B content in the brain might participate in antidepressant action [11].

The expression of S100B appears to be affected by aging. In an analysis of postmortem human brain tissue, the number of S100B-positive cells and the tissue content of S100B mRNA and S100B protein was shown to increase with advancing age [15]. In the rat brain, aging was also accompanied by increased brain levels of S100B [16]. However, significant regional differences in these aging effects in the rat brain were observed. Thus, an aging-associated increase of S100B content was observed in the cerebral cortex but not in the cerebellum or in the brain stem [16]. In this study, the hippocampus was not analyzed.

The brain content of S100B was also investigated in senescence acceleration-prone mice (SAMP). It was found that SAMP mice have increased S100B in the hippocampus and cerebral cortex compared to control mice [17]. Nevertheless, it is not clear whether aging interferes with the ability of fluoxetine to increase S100B content in the brain.

In the present study, we analyzed the hippocampal S100B content of young (2 month) and old (24 month) mice, and we investigated whether the stimulatory action of fluoxetine on hippocampal S100B content, which had been previously observed in young rats, was also present in old mice.

MATERIALS AND METHODS

Animals and drug treatment: Experiments were performed on young (2-month-old, $n=6$) and old (24-month-old, $n=15$) C57BL/6 mice obtained from Harlan, Inc. (Indianapolis, IN). They were housed under conditions of controlled temperature ($23 \pm 2^{\circ}C$) and illumination (13:11 h light:dark; lights off 18.00 h). Mice were injected with 10 mg/kg fluoxetine (Sigma, St Louis, MO) or its vehicle (dimethylsulfoxide; Sigma, St Louis, MO; 1% in saline) daily for 2 weeks. All groups received treatments at 10.00 h. The experimental protocol was approved by the Institutional Animal Care Committee.

S100B Western immunoblotting: To quantify the levels of hippocampal S100B, mice from both groups were sacrificed 24 h after the 14th injection. Their hippocampi were dissected out, homogenized, and processed for Western immunoblotting with a commercially available S100B antibody as described earlier [14]. Briefly, the hippocampal tissue was homogenized in a homogenizing buffer in the presence of protease inhibitors. After reading the protein content of the tissue homogenate, 10 and 20 μg samples and gel loading solution were mixed and boiled for 3 min. They were loaded onto a 15% (w/v) acrylamide gel and run using the Mini Protean II gel apparatus (Bio-Rad, Hercules, CA). The proteins were subsequently transferred electrophoretically to an ECL nitrocellulose membrane (Amersham, Piscataway, NJ) using the Mini TransBlot transfer unit (Bio-Rad, Hercules, CA) at 150 mA constant current for 2 h. The blots were incubated with rabbit anti-S100B antibody (1:10000, Research Diagnostics Inc., Flanders, NJ) in 5% (w/v) powdered nonfat milk in TBST, 2 ml NP-40, and 0.02% (w/v) SDS (pH 8.0) overnight. The blots were then washed and incubated with horseradish-peroxidase-linked secondary antibody (anti-rabbit IgG; 1:1000; Amersham, Piscataway, NJ, USA) for 4 h at room temperature, processed with an ECL kit, and exposed to Hyperfilm ECL (Amersham, Piscataway, NJ, USA). To normalize the signal for S100B protein, the presence of noninducible β-actin protein was measured on the same blot using a mouse monoclonal antibody against the β-actin (1:5000; Sigma, St. Louis, MO, USA). The optical densities on film of the S100B bands were corrected by the optical density of the corresponding β-actin bands using the Loats Image Analysis System (Loats Associates, Inc., Westminster, MD)] and their ratios were calculated.

S100B Immunoassay: The amount of S100B in hippocampal tissue was measured using the S100B immunoassay kit, CanAg S100BB EIA (Polymedco Inc., Cortlandt Manor, NY). The tissue samples were homogenized in 250 μl 50 mM Tris–HCl buffer (pH 7.5) containing 5 mM $MgCl_2$ and centrifuged at 4°C at 20 000 r.p.m. for 1 h. A 50 μl aliqout of each supernatant were run in duplicate following the manufacturer's manual. The optical density of samples was read at 405 nm, and the concentration of S100B was calculated from a standard curve that was run along with the samples.

The S100B levels were corrected for the protein content of the samples. Protein levels were measured following a standard Bradford protein assay procedure.

Statistical analysis: All results (mean ± s.e.m.) were analyzed by Student's *t*-test. Significance was accepted at $p < 0.05$.

RESULTS

The hippocampal S100B contents of young and old mice were compared using two methods, Western immunoblotting assay and by a commercially available immunoassay kit. Both methods revealed significantly higher S100B content in old versus young hippocampus (Fig. 1). The protracted systemic administration of fluoxetine to old mice resulted in a further increase in hipoocampal S100B (Fig. 2).

DISCUSSION

Our findings provide further evidence that aging leads to an increase of brain S100B content in mammals. Previous studies in rats suggested that the aging-associated increase of S100B is region specific. Thus, an increase was observed in the cerebral cortex but not in the cerebellum and the brain stem [16]. However, these earlier studies did not examine hippocampal S100B. Our data indicate that aging-associated changes in hippocampal S100B resemble changes of cortical but not cerebellar or brain stem S100B. This finding is important because hippocampal S100B has been implicated in Alzheimer's disease symptomatology [10] and in the antidepressant action of drugs such as fluoxetine [12].

Evidence of increased brain S100B in elderly subjects and also in postmortem tissue obtained from Alzheimer's patients [15] led to the proposal that in spite of the neurotrophic action of S100B, an over-expression/accumulation of this protein in the brain might be responsible for the progression of Alzheimer's pathology. This hypothesis proposes that there is a critical threshold above which S100B could become a pathogenic factor [15]. On the other hand, findings of increased hippocampal S100B following chronic

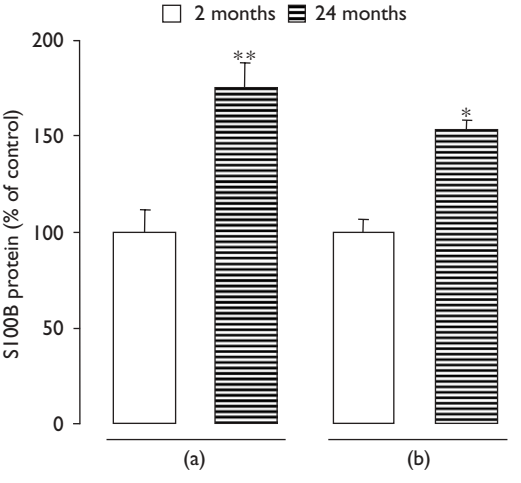

Fig. 1. Hippocampal SI00B content is greater in old than in youg mice. The hippocampi were homogenized and analyzed by Western immunoblotting (**a**) and by an immunoassay (**b**). Open bars, young mice (2 months); filled bars, old mice (24 months). Results are expressed as a percentage of the corresponding 2-month values and are mean ± s.e.m. (*$p < 0.05$, **$p < 0.01$ Student's *t*-test).

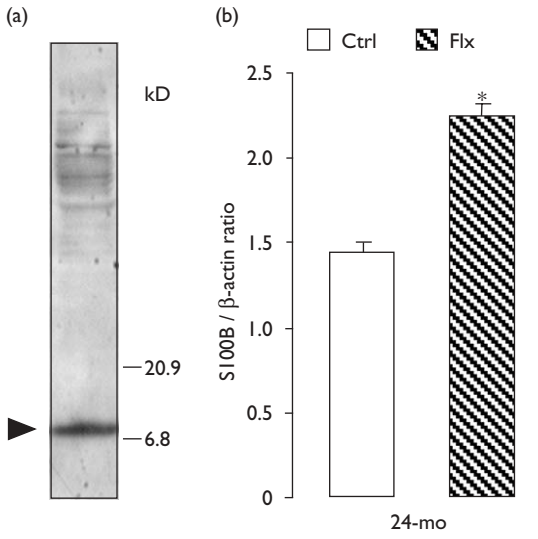

Fig. 2. Fluoxetine increases hippocampal S100B in 24-month-old mice. Mice were treated for 2 weeks daily with 10 mg/kg fluoxetine, i.p., or with its vehicle. Their hippocampi were homogenized, proteins measured, and two dilutions from each sample (20 and 10 μg protein/lane) were run on 15% acrylamide gels, blotted, and probed with S100B (an example is shown in (**a**); the arrowhead points to the S100B band; the size markers are indicated on the right) and β-actin antibodies, and the average S100B/β-actin ratios were calculated. (**b**) open bars, control (Ctrl); filled bars, fluoxetine (Flx). Results are mean ± s.e.m (*$p < 0.05$; Student's *t*-test).

fluoxetine administration [14] led to the proposal that the neurotrophic action of S100B could contribute to antidepressant activity [18]. Both these proposals require further direct testing. Nevertheless, our findings indicate that the stimulatory action of fluoxetine on hippocampal S100B is not restricted to young subjects, it also occurs in old animals that have already demonstrated elevated levels of S100B. If aging-associated elevation of brain S100B is responsible for the increments of Alzheimer's pathology, an additional increase of S100B by antidepressants such as fluoxetine might be contra-indicated in these patients. Nevertheless, antidepressants are being considered for treatment of depressive symptoms in Alzheimer's patients, and some of these treatments might have a cognitive enhancing effect [19].

In vivo measurements of S100B in humans are restricted to peripheral samples, typically blood. It has been proposed that serum S100B levels can be used as a marker of blood–brain disruption [20]. There is some evidence that the blood–brain barrier might be disrupted in depressive illness. For example, Niklasson and Agren [21] proposed that damaged blood–brain barrier permeability may contribute to the modulation and symptomatology of depressive illness. For example, patients with unipolar depression and an impaired blood–brain barrier had a more protracted onset of their illness and were more suicidal than those with a less permeable blood–brain barrier [21]. On the other hand, studies with rats demonstrated that antidepressant treatment can significantly reduce cold-lesion-triggered increased blood–brain permeability [22]. Preliminary studies in depressed patients are indicative of a possible disruption of blood–brain permeability in this illness and suggest that antidepressants might influence such a disturbance. Thus, Schroeter *et al*. [23] reported that the serum content of S100B is elevated in patients with major depression (20 subjects) compared with healthy controls (12 subjects). These authors observed that antidepressant treatment reduced both S100B content and the severity of depressive symptoms.

CONCLUSION

In this study, we found that the hippocampal content of a neurotrophic cytokine S100B is elevated in old (24 month) compared to young (2 month) mice. Treatment of old mice with the antidepressant fluoxetine for 2 weeks further elevated the hippocampal S100B content. Currently, it is believed that elevated brain levels of S100B might be associated with the progression of Alzheimer's disease. If so, the additional increase of brain S100B in elderly subjects with antidepressant treatment should be considered when these drugs are administered to Alzheimer's patients.

REFERENCES

1. Heizmann CW. *Neurochem Res* **24**, 1097–1100 (1999).
2. Donato R. *Int J Biochem Cell Biol* **33**, 637–668 (2001).
3. Selinfreund RH, Barger SW, Pledger WJ and Van Eldik LJ. *Proc Natl Acad Sci USA* **88**, 3554–3558 (1991).
4. Whitaker-Azmitia PM, Murphy R and Azmitia EC. *Brain Res* **528**, 155–158 (1990).
5. Ahlemeyer B, Beier H, Semkova I *et al*. *Brain Res* **858**, 121–128 (2000).
6. Barger SW, Van Eldik LJ and Mattson MP. *Brain Res* **677**, 167–170 (1995).
7. Huttunen HJ, Kuja-Panula J, Sorci G *et al*. *J Biol Chem* **275**, 40096–40105 (2000).
8. Marshak DR. *Prog Brain Res* **86**, 169–181 (1990).
9. Reeves RH, Yao J, Crowley MR *et al*. *Proc Natl Acad Sci USA* **91**, 5359–5363 (1994).
10. Mrak RE and Griffin WS. *Neurobiol Aging* **22**, 915–922 (2001).
11. Manev H and Manev R. *Med Hypotheses* **58**, 83–84 (2002).
12. Manev H, Uz T and Manev R. *J Affect Disord* **75**, 59–64 (2003).
13. Haring JH, Hagan A, Olson J and Rodgers B. *Brain Res* **631**, 119–123 (1993).
14. Manev R, Uz T and Manev H. *Eur J Pharmacol* **420**, R1–R2 (2001).
15. Sheng JG, Mrak RE, Rovnaghi CR *et al*. *Neurobiol Aging* **17**, 359–363 (1996).
16. Kato K, Suzuki F, Morishita R *et al*. *J Neurochem* **54**, 1269–1274 (1990).
17. Griffin WST, Sheng JG and Mrak RE. *Neurobiol Aging* **19**, 71–76 (1998).
18. Manev H and Manev R. *J Psychiatr Res* **35**, 347–350 (2001).
19. Tune LE. *Depress Anxiety* **8**(**Suppl. 1**), 91–95 (1998).
20. Kapural M, Krizanac-Bengez LJ, Barnett G *et al*. *Brain Res* **940**, 102–104 (2002).
21. Niklasson F and Agren H. *Biol Psychiatry* **19**, 1183–1206 (1984).
22. Raymond JJ, Robertson DM, Dinsdale HB and Nag S. *Can J Neurol Sci* **11**, 445–451 (1984).
23. Schroeter ML, Abdul-Khaliq H, Diefenbacher A and Blasig IE. *Neuroreport* **13**, 1675–1678 (2002).

Acknowledgements: This study was supported in part by a 2001 UIC CRB award to R.M.