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Fluoxetine increases the content of neurotrophic protein S100β in the rat hippocampus

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Abstract

Recent studies indicate that a protracted daily administration of the antidepressant fluoxetine to adult rats increases cell proliferation/neurogenesis in the hippocampus. It has been hypothesized that this action of fluoxetine might be mediated by neurotrophic factors. We hypothesized that glial S100β could be such a factor, and using quantitative Western immunoblotting, we investigated the effect of a 21-day treatment of rats with fluoxetine (5 mg/kg), and found that fluoxetine increases the content of hippocampal S100β.

Keywords: Neurogenesis; Antidepressant; Glia

Antidepressants and, particularly, the selective serotonin reuptake inhibitor fluoxetine, increase neurogenesis in the adult mammalian brain, e.g. in the hippocampus (Malberg et al., 2000; Manev et al., 2001). Based on these findings, a novel theory of depression has been formulated that proposes a deficiency in adult brain neurogenesis as the pathobiological basis of depression. The exact mechanisms of antidepressant-triggered neurogenesis in the adult brain are not clear and are being actively investigated. For example, Malberg et al. (2000) suggested that both the cAMP cascade and brain-derived neurotrophic factor (BDNF), which they found are upregulated by antidepressant treatment, may play a role in the regulation of neurogenesis. Moreover, intraventricular infusion of BDNF was capable of increasing neurogenesis in the adult olfactory bulb (Zigova et al., 1998). Another possibility is that insulin-like growth factor (IGF-1), a growth-promoting peptide hormone that has neurotrophic properties, increases the proliferation and survival of neurons in the adult rat, although no data are available on the effects of antidepressants on IGF-1. In this work, we hypothesize that yet another neurotrophic factor, S100β, may play a crucial role in regulating adult neurogenesis and may be a target for the action of antidepressant drugs.

S100β is a small acidic Ca^{2+}-binding neurotrophic protein that is highly concentrated in the vertebrate nervous system; it is produced in astroglia and can increase cell proliferation when released (Selinfreund et al., 1991). The content of S100β in the central nervous system (CNS) appears to be regulated by serotonin and antidepressants (e.g., fluoxetine). For example, stereological immunohistochemical analysis revealed a direct relationship between the expression/distribution of S100β immunoreactivity and the levels of serotonin in rat hippocampus. Thus, when serotonin synthesis was reduced, S100β immunoreactivity was also reduced. The opposite reaction was observed in rats treated with fluoxetine (Haring et al., 1993). Here, we used quantitative Western immunoblotting to measure the effects of a protracted (3 weeks) treatment of rats with fluoxetine on the hippocampal content of S100β. We found that this treatment schedule was effective in increasing hippocampal cell proliferation (Manev et al., 2001).

Brown–Norway rats (217 BNRIJ; Harlan, Indianapolis, IN, USA) weighing 240–260 g were housed three per cage under a 14 h light/10 h dark cycle (darkness commenced at 18:00). They were injected with fluoxetine (5 mg/kg; RBI; F-132) or its vehicle (1% dimethylsulfoxide, DMSO, in saline) 1 h prior to darkness. Rats were sacrificed 18 h after the 21st injection (Manev et al., 2001), their hippocampi were dissected out, homogenized, and processed for Western immunoblotting with S100β antibody (rabbit anti-bovine; Research Diagnostic, Flanders, NJ, USA) (1:10,000; 4°C, overnight) and anti-rabbit immunoglobulin G (IgG) (1:2000 for 5 h) as the secondary antibody. We

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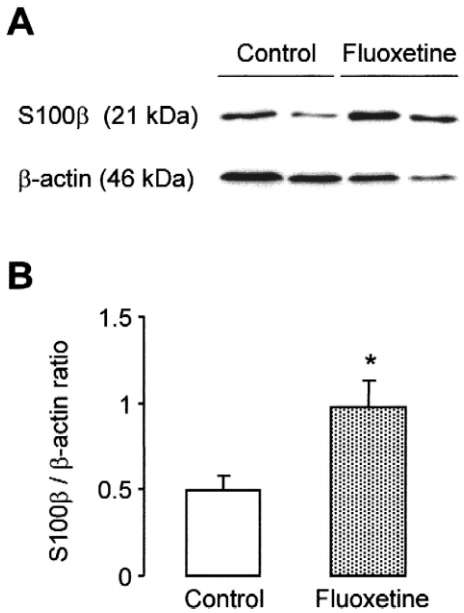

Fig. 1. Stimulatory effect of fluoxetine on S100β content in the rat hippocampus. Rats were treated for 21 days daily with 5 mg/kg fluoxetine (intraperitoneal injections; $n = 6$) or with its vehicle (control; $n = 5$). Their hippocampi were homogenized, proteins were measured, and two dilutions from each sample (20 and 10 μg protein/lane) were run on 15% acrylamide gels, blotted, and probed with S100β and β-actin antibodies (an example is shown in A). Each hippocampal sample was analyzed at least twice and the average S100β/β-actin ratios were calculated. Results (B) are mean ± S.E.M. ($P < 0.01$; Student's *t*-test).

simultaneously measured β-actin immunoreactivity using monoclonal primary antibody (1:3000 for 2 h; Sigma, St. Louis, MO, USA) and anti-mouse IgG (1:3000 for 2 h) as the secondary antibody. The optical densities of the bands on the film (Fig. 1A) were quantified using the Loats Image Analysis System (Westminster, MD, USA). The optical density of each S100β band (21 kDa) was corrected by the optical density of the corresponding β-actin band (46 kDa) and the results are expressed as S100β/β-actin ratios.

We found that fluoxetine treatment significantly increased the hippocampal content of S100β protein (Fig. 1B). Thus, we propose that S100β might be considered a mediator of the recently discovered stimulatory action of antidepressant drugs on hippocampal cell proliferation (Malberg et al., 2000; Manev et al., 2001). Although S100β primarily stimulates glial proliferation, its putative relevance for neurogenesis is that glial precursors can also generate neuronal cells and thus promote neurogenesis (Noctor et al., 2001). Alternatively, S100β may also exert a neuroprotective/anti-apoptotic influence on CNS neurons (Ahlemeyer et al., 2000; Huttunen et al., 2000) and might also contribute to neurogenesis by reducing the rate of apoptosis of immature cells. Further studies would clarify the putative role of glia in mediating the action of antidepressant drugs.

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