

Antidepressant Treatments Induce the Expression of Basic Fibroblast Growth Factor in Cortical and Hippocampal Neurons

ALESSANDRA MALLEI, BITAO SHI, and ITALO MOCCHETTI

Department of Neuroscience, Georgetown University Medical Center Washington DC; and Department of Toxicology, University of Cagliari, Cagliari, Italy

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ABSTRACT

New experimental evidence suggests that the mechanism of action of antidepressants includes the induction of neurotrophic factor synthesis in selected brain areas. The present study is aimed at establishing whether prolonged antidepressant treatments increase the expression of basic fibroblast growth factor (FGF2), a polypeptide growth factor that has a broad neurotrophic activity in the adult central nervous system. Rats received a single dose or long-term (3 weeks) administration of desipramine (DMI), fluoxetine (FLU), and mianserin (MIA), then were sacrificed at 5 and 24 h after the last injection. RNase protection assay and Western blot analysis revealed that all antidepressant drugs elicited an anatomically specific increase in FGF2 mRNA and protein. The

increase in FGF2 mRNA after a single injection was seen only at 5 h after the injection and was restricted to the entorhinal cortex, whereas the effect of the long-term treatments lasted up to 24 h and occurred in the entire cortex and hippocampus. Immunohistochemical analysis of FGF2 immunoreactivity was carried out to investigate which cell types responded to the antidepressant treatments. DMI and MIA increased FGF2 proteins predominantly in neurons of layer V throughout the cerebral cortex and in some neurofilament-positive cells of the hippocampus. FLU increased FGF2 immunoreactivity mainly in neurofilament-positive cells of the hippocampus. These findings may explain the therapeutic efficacy of antidepressants in affective disorders.

The mechanism of action of antidepressant drugs and their effectiveness in psychiatric illness remain poorly understood. For almost 3 decades, antidepressants have been known for their ability to preferentially inhibit the uptake of norepinephrine and serotonin and therefore to affect monoaminergic function. However, this mechanism of action alone may not account for their therapeutic efficacy, considering that clinical responses to antidepressant drugs require long-term administration.

Recent studies have shown that antidepressant treatments increase the activity of transcription factors (Nibuya et al., 1996), suggesting that the mechanism of action of antidepressants may include alteration of expression of genes involved in synaptic plasticity. In particular, antidepressants increase the expression of brain-derived neurotrophic factor (BDNF) (Nibuya et al., 1995), a member of the neurotrophin family of neurotrophic factors that affect the survival and differentiation of neurons expressing distinct neurotrophin receptors, including serotonergic neurons. In fact, BDNF promotes sprouting of injured serotonergic neurons and causes a

substantial increase in serotonergic axon density in the neocortex (reviewed in Koliatsos et al., 2001). In addition, BDNF knock-out mice show impaired serotonergic function (Lyons et al., 1999), indicating that BDNF might be a potential therapy for psychiatric illness characterized by alterations of serotonergic activity (Duman et al., 1997). However, some affective disorders seem also to be related to a functional deficit of other monoamines, particularly norepinephrine. Thus, other neurotrophic factors may be necessary to improve the monoaminergic impairment observed in affective disorders.

Activation of the central noradrenergic system, as obtained by activation of β_2 -adrenergic receptors (Follesa and Mocchetti, 1993; Hayes et al., 1995) or experimental electroshock (Follesa et al., 1994; Gall et al., 1994) has been shown to increase the synthesis of basic fibroblast growth factor (FGF2) in selected areas of the rat brain. FGF2 is another neurotrophic factor present in the adult central nervous system (CNS) that has been initially characterized by its ability to exert trophic effects on CNS neurons in vitro (Morrison et al., 1986; Walicke, 1988), and to enhance survival of neurons in vivo after injury or ischemia (Gomez-Pinilla et al., 1992; Peterson et al., 1996; Kawamata et al., 1997; Teng et al.,

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ABBREVIATIONS:BDNF, brain derived neurotrophic factor; FGF2, basic fibroblast growth factor; CNS, central nervous system; DMI, desipramine; FLU, fluoxetine; MIA, mianserin; NGF, nerve growth factor; FGF1, acidic fibroblast growth factor; IR, immunoreactivity; ANOVA, analysis of variance; LMM, low molecular mass; HMM, high molecular mass.

1998) or neurotoxins (Frim et al., 1993). Moreover, FGF2 promotes gliogenesis and neurogenesis when added to cultures of precursor cells from various brain areas (Vescovi et al., 1993; Qian et al., 1997) or in vivo in developing rats (Raballo et al., 2000) and adult rats (Shihabuddin et al., 1997; Yoshimura et al., 2001). These data raise the prospect that FGF2 may prove to be a therapeutic strategy for minimizing the number of neurons critically impaired in affective disorders. This hypothesis, however, could be supported only if antidepressants increase the expression of FGF2 in the brain. In the present study, we have used three antidepressant drugs characterized by different pharmacological profiles, to test whether antidepressants increase the availability of FGF2. These drugs include desipramine (DMI), a norepinephrine-selective reuptake inhibitor, fluoxetine (FLU), a serotonin-selective reuptake inhibitor, and mianserin (MIA), an atypical antidepressant. We report that all antidepressants increase FGF2 synthesis in selected brain areas.

Materials and Methods

Animal Treatment and Tissue Preparation. Adult male Sprague-Dawley rats (180–250 g; Taconic, Germantown, NY) were housed in a temperature-controlled environment with a 12-h light/dark cycle and access to food and water ad libitum. Animals received either a single i.p. injection (acute) or daily i.p. injections for 21 days (long-term) of saline, DMI (15 mg/kg; Sigma, St Louis, MO), FLU (5 mg/kg; Eli-Lilly, Indianapolis, IN), or MIA (10 mg/kg; Sigma). Rats (total $n = 9$ for each group) were sacrificed by decapitation for biochemical determinations. Frontal and entorhinal cortex, hippocampus, and striatum were dissected on ice and stored at -70°C until processed. For histological analysis, rats were perfused intracardially with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, for 20 min. The brain was removed and postfixed in the same fixative for 2 h, then transferred into the buffered graded sucrose (10, 20, and 30%).

RNAse Protection Assay. Extraction of RNA and RNase protection assay was carried out as described previously (Follesa and Mocchetti, 1993; Mocchetti et al., 1996a). In brief, levels of FGF2 mRNA were determined using a ^{32}P -labeled 524-base probe that includes the 477 bases of FGF2 cRNA and 47 bases of the plasmid polylinker region (Shimasaki et al., 1988). For nerve growth factor (NGF) mRNA determination, a ^{32}P -labeled NGF RNA probe was generated from plasmid BSRNGF (Whittemore et al., 1988) that contains a 771-base portion of the rat NGF cDNA. Cyclophilin cRNA was used as an internal reference to standardize variations in extraction of RNA from tissue samples (Mocchetti et al., 1996a,b). RNA hybridization was carried out at 50°C overnight. RNA was digested with RNase A (1 U/ml) and T1 (200 U/ml) for 30 min at 35°C . Protected fragments were separated on a 5% polyacrylamide/urea sequencing gel. The gel was dried and the FGF2 protected fragments was visualized by autoradiography on X-ray film using Chronex Quanta III intensifying screen. FGF2 and NGF mRNA content was calculated by measuring the peak densitometry area of the autoradiograph analyzed by a densitometer (Bio-Rad GS-710; Bio-Rad Laboratories, Hercules, CA) normalized by the peak densitometry area of the cyclophilin autoradiograph band.

Detection of FGF2 by Western Blot Analysis. Analysis of FGF2 levels was carried out by Western blot as described previously (Mocchetti et al., 1996a,b). In brief, brain tissue extracts were prepared by homogenization in 10 volumes (w/v) of ice-cold extraction buffer [20 mM Tris buffer, pH 7.4, 2 mM EDTA, 1% Nonidet P-40, 2 M NaCl, 1 mM phenylmethylsulfonyl fluoride, 1 $\mu\text{g}/\text{ml}$ aprotinin, 1 $\mu\text{g}/\text{ml}$ leupeptin, 1 $\mu\text{g}/\text{ml}$ pepstatin (all from Sigma)]. Samples were centrifuged for 20 min at 15,000g and protein content was measured

in the supernatant by the Bradford Coomassie Blue colorimetric assay (Bio-Rad). Fractions of the supernatant (400 μl) were added to a tube containing 50 μl of heparin-Sepharose CL-6B (Amersham Biosciences, Piscataway, NJ) slurry (100 mg swollen in 1-ml Tris/EDTA buffer containing 0.6 M NaCl) and rocked overnight at 4°C . The heparin-sepharose was centrifuged at 13,000g for 5 min, and the pellet washed three times with 0.6 M NaCl, 10 mM Tris HCl, pH 7.4. The final pellet was boiled in loading buffer (2% SDS, 100 mM dithiothreitol, 10% glycerol, 0.25% bromophenol blue) and separated in a 15% SDS-polyacrylamide gel. Prestained molecular mass markers (Bio-Rad), recombinant human FGF2, and acidic FGF (FGF1) (Collaborative Research, Bedford, MA) were run simultaneously. Proteins were electrophoretically transferred onto nitrocellulose filter. Immunostaining of blotted proteins was carried out using a rabbit FGF2 polyclonal antibody (Chemicon International Inc., Temecula, CA). Blots were analyzed using the enhanced chemiluminescence system (Amersham).

Immunocytochemistry. Analysis of immunoreactivity (IR) was carried out as described previously (Shi et al., 1998). In brief, serial sections (30 μm) were prepared from brains and incubated with monoclonal mouse anti-FGF2 (Type II antibodies; Upstate Biotechnology Inc., Lake Placid, NY) at a dilution 1:150 in Tris-HCl saline buffer at 4°C for 48 h. Sections were then incubated with biotinylated horse anti-mouse IgG (dilution 1:200) at room temperature for 2 h. Immune complexes were detected with the Vectastain Elite ABC kit (Vector Labs, Burlingame, CA). FGF2 antigen was visualized using nickel-enhanced diaminobenzidine.

For double staining, sections were incubated with monoclonal mouse anti-neurofilament SMI 311 (a cocktail of monoclonal IgG1 and IgM antibodies to nonphosphorylated neurofilaments; 1:1000; Sternberger Monoclonals Inc., Lutherville, MD) at 4°C for 48 h, followed by incubation in goat anti-mouse IgG (Sternberger Monoclonals, Inc.) at room temperature for 2 h. Sections were then incubated with mouse peroxidase anti-peroxidase complex at room temperature for 2 h and the immune complexes detected with diaminobenzidine. Sections of saline and treated animals were mounted on the same slide and were processed identically.

Statistical Analysis. Normality test was used to verify the homogeneity of the values. Differences among means were evaluated by ANOVA. When treatments elicited significant changes, significance was determined by Dunnett's test (for comparing treatment groups with control group) and Sheffé's test (for multiple comparisons).

Results

Acute Antidepressant Administration Elicits an Anatomy-Specific Increase in FGF2 mRNA. To determine whether antidepressants alter FGF2 mRNA levels in the brain, rats received an acute i.p. administration of saline, DMI (15 mg/kg), or FLU (5 mg/kg) and were sacrificed 5 and 24 h after the injection. FGF2 mRNA was determined in the hippocampus, striatum, and frontal and entorhinal cortex. RNase protection assay revealed an anatomical-specific increase in FGF2 mRNA levels by the treatments. By 5 h, DMI and FLU increased FGF2 mRNA levels in entorhinal cortex (Fig. 1). DMI was more potent than FLU (Fig. 1). None of the antidepressants significantly increased FGF2 mRNA in the frontal cortex, hippocampus or striatum (Fig. 2). No increase was observed at 24 h in any region examined (data not shown).

Effect of Chronic Treatment on FGF2 mRNA. The therapeutic activity of antidepressants is usually seen after a prolonged administration. To examine whether DMI and FLU change FGF2 mRNA levels even after a longer treatment, rats received saline, DMI, or FLU once daily for 21

days and were sacrificed 5 and 24 h after the last injection. RNase protection assay also revealed that the long-term treatment elicited an anatomically selective induction of FGF2 mRNA depending upon the antidepressant used. In fact, DMI increased FGF2 mRNA content in the entorhinal and frontal cortex up to 24 h after the last injection (Fig. 3). FLU, instead, failed to change significantly FGF2 mRNA levels in these brain areas (Fig. 3). Other brain areas were examined; in the hippocampus, both DMI and FLU increased FGF2 mRNA (Fig. 3). None of the antidepressants increased FGF2 mRNA in the striatum (Fig. 3).

To examine the specificity of the antidepressant treat-

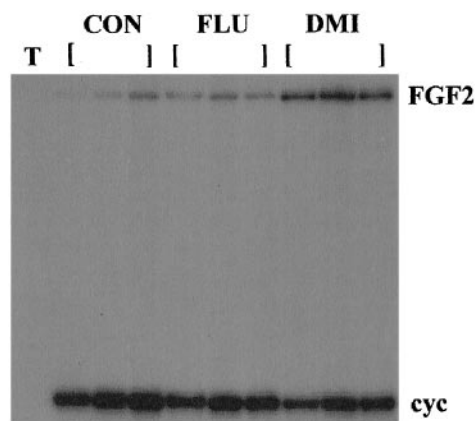


Fig. 1. RNase protection assay of FGF2 mRNA levels in the entorhinal cortex after acute antidepressant treatment. Rats received saline (CON), FLU, or DMI and were sacrificed 5 h after the injection. Entorhinal cortex was dissected, total RNA extracted and analyzed, as described previously, using FGF2 and cyclophilin (cyc) probes (Mocchetti et al., 1996a,b). FGF2 and cyclophilin protected fragments are indicated. T, tRNA.

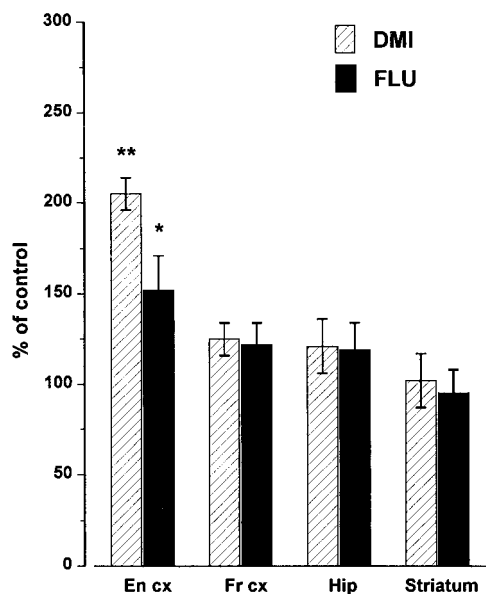


Fig. 2. DMI and FLU increase FGF2 mRNA levels in specific brain areas. Rats received saline, DMI, or FLU and were sacrificed 5 h after the injection. Levels of FGF2 mRNA were analyzed in the indicated brain areas by RNase protection assay as described under *Materials and Methods*. En cx, entorhinal cortex; Fr cx, frontal cortex; Hip, hippocampus. Quantitation of FGF2 mRNA was carried out by densitometric scanning of the protected fragment and normalized by cyclophilin mRNA as described previously (Mocchetti et al., 1996a,b). Data, expressed as percentage of control (saline-treated rats), are the mean \pm S.E.M. of six independent samples. *, $p < 0.05$; **, $p < 0.01$ versus control, ANOVA and Dunnett's test.

ments used in this study, rats received the atypical antidepressant MIA (10 mg/kg) for 21 days. MIA increased FGF2 mRNA in both entorhinal cortex and hippocampus (Fig. 3). Thus, at least from the anatomical point of view, the effect of MIA seems to be similar to that of DMI. To further examine specificity of the treatments, we determined NGF mRNA levels in the same tissue extracts used to measure FGF2 mRNA. RNase protection assay revealed that long-term treatments with DMI, MIA, or FLU increase NGF mRNA in the entorhinal cortex but not in the frontal cortex or hippocampus (Fig. 4), supporting the hypothesis that antidepressants target various neurotrophic factors in different brain areas.

Antidepressant Administration Increases FGF2 Proteins. Antidepressant agents may affect FGF2 synthesis; therefore, changes in FGF2 mRNA levels should be followed by increases in FGF2 proteins. To test this hypothesis, we determined FGF2 proteins by Western blot analysis. Rats received saline, DMI, FLU, or MIA for 21 days and were sacrificed 5 h after the last injection. Cortical and hippocampal extracts were prepared and analyzed with an FGF2 monoclonal antibody that does not show cross-reactivity with FGF1 (Fig. 5). In both hippocampal (Fig. 5) and cortical extracts (heparin-bound material) of saline-treated rats, this antibody recognized at least three major FGF2-like IR forms, a low molecular mass (LMM) 18-kDa band, and two higher molecular mass (HMM) forms of ~21 to 22 kDa (Fig. 5). The

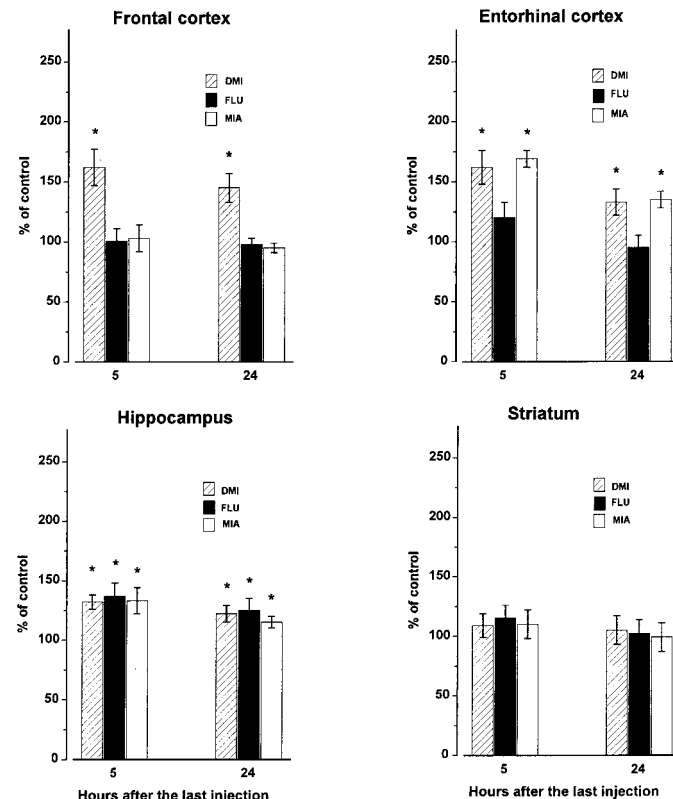


Fig. 3. Chronic treatments with antidepressants induce FGF2 mRNA. Rats received saline, DMI, FLU, or MIA daily for 21 days and were sacrificed 5 and 24 h after the last injection. Levels of FGF2 mRNA were analyzed in the indicated brain areas by RNase protection assay as described in Figs. 1 and 2. Data, expressed as percentage of control (saline-treated rats), are the mean \pm S.E.M. of three independent experiments ($n = 3$ each experiment). *, $p < 0.05$ versus control, ANOVA and Dunnett's test.

22-kDa form was more abundant than the other two forms, as shown previously (Mocchetti et al., 1996a,b). In hippocampal extracts from DMI and FLU-treated rats, the intensity of the HMM and LMM forms was increased above the levels of control animals (Fig. 5). Semiquantitative analysis of these forms by densitometry revealed that DMI changes FGF2 isoforms in both the cortex and hippocampus, whereas FLU was effective in the hippocampus only (Fig. 6). MIA increased FGF2 levels in both hippocampus and cortex (Fig. 6), overall supporting a correlation between increases in mRNA and protein content.

Antidepressants Increase FGF2-IR. To gain additional information on the anatomical specificity of FGF2 expression after antidepressant treatments, we examined FGF-IR by immunohistochemistry. Rats received a systemic injection of saline, DMI, MIA, and FLU daily for 21 days and were sacrificed 5 h after the last injection, then serial coronal sections (30 μ m) were prepared throughout the cerebral cor-

tex and hippocampus. In saline-treated rats, in both the frontal (Fig. 7, A and C) and entorhinal cortex (Fig. 7, E and G), FGF2-IR was confined to small cells indicative of glial cells (Fig. 7, C and G, arrowheads). Analysis of FGF2-IR in antidepressant-treated rats showed a completely different profile of distribution. In fact, both the frontal and entorhinal cortex of DMI-treated rats showed intense FGF2-IR, mostly in layer V (Fig. 7, B and F) in cells with a neuronal morphology (Fig. 7, D and H, arrows). The increase in FGF2-IR by DMI was observed in several portions of the cortex and also in the hippocampus (data not shown). A similar increase was observed in sections from MIA-treated rats (data not shown). Instead, cortical sections from FLU-treated rats did not show any appreciable induction of FGF2-IR (data not shown) supporting the data obtained by Western blot analysis.

Cells of the hippocampus were also positive for FGF2-IR. In control rats, FGF-IR was localized in small cells (Fig. 8, A, C, E, G, and I) throughout the hippocampus, suggesting glial

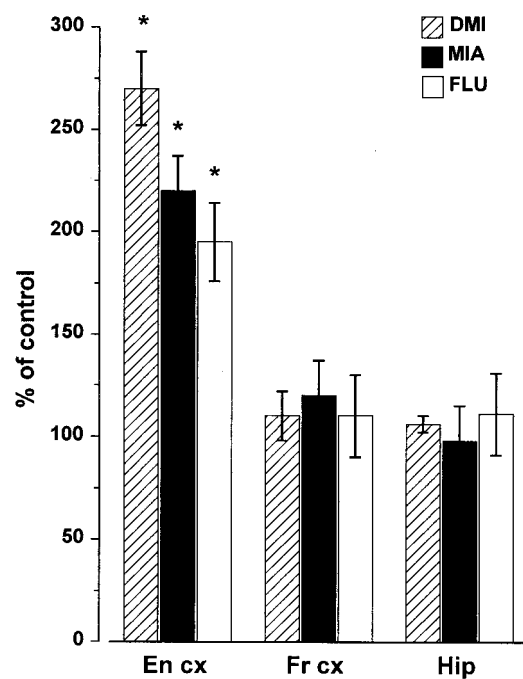


Fig. 4. Antidepressants increase NGF mRNA in the entorhinal cortex. Tissue extracts from saline, DMI, FLU, and MIA-treated rats were analyzed for NGF mRNA in the indicated brain areas by RNase protection assay. Data, expressed as percentage of control (saline-treated rats), are the mean \pm S.E.M. of six independent determinations. *, $p < 0.01$ versus control, ANOVA and Dunnett's test.

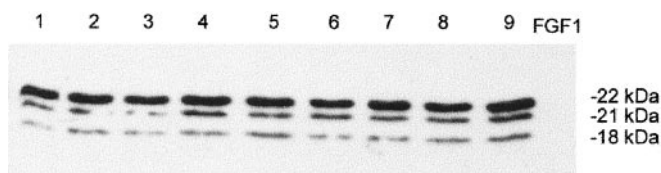


Fig. 5. Western blot analysis of FGF2-IR. Animals received saline, DMI, or FLU for 21 days and were sacrificed 5 h after the last injection. Brain areas were dissected, lysates prepared as described under *Materials and Methods*, then heparin binding proteins were enriched by affinity chromatography. Example of Western blot analysis of hippocampal extracts (one seventh of the total extract) using FGF2 monoclonal antibody (dilution, 1/500). Lanes 1 to 3, saline-treated rats; lanes 4 to 6, DMI-treated rats; lanes 7 to 9, FLU-treated rats. FGF1, 10 ng of human recombinant FGF1.

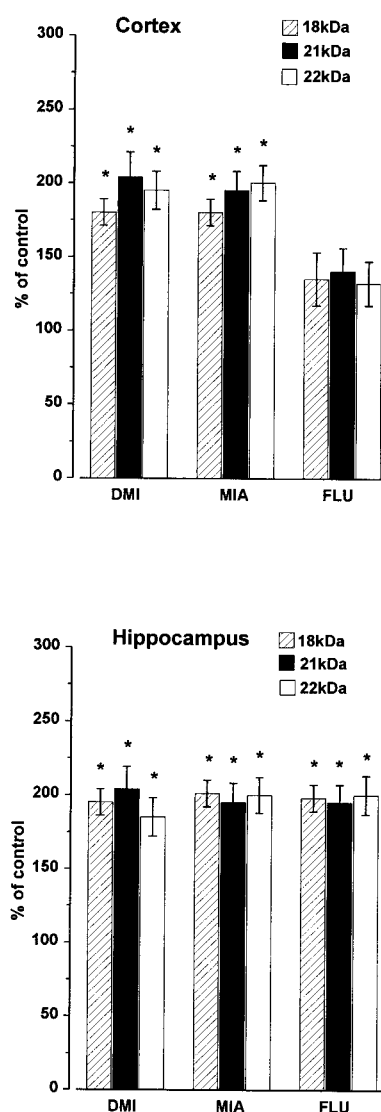


Fig. 6. Effect of antidepressants on FGF2 isoforms. Cortical and hippocampal lysates from saline, DMI-, MIA-, and FLU-treated rats were analyzed by Western blot as described in Fig. 5. The arbitrary levels of FGF2-IR isoforms were calculated by densitometry of the IR bands. Data, expressed as percentage of control, are the means \pm S.E.M. of six independent samples. *, $p < 0.01$ versus control, ANOVA and Dunnett's test.

cells. Sections from DMI-treated rats showed increased FGF2-IR in large cells with a neuronal morphology in the stratum oriens and pyramidal cell layer of the CA1 region (Fig. 8, B and D). Large cells in the CA2 layer were also positive for FGF2 (Fig. 8, H and J). Similar anatomical distribution of FGF2-IR was observed in rats treated with MIA and FLU (data not shown).

To confirm the neuronal localization of FGF2-IR in neurons, sections from rats treated with saline, DMI, and FLU rats were double-stained with FGF2 and neurofilament antibodies. In cortical sections from control rats, FGF2-IR was scattered throughout the cerebral cortex and seemed localized in the nucleus of small ($\sim 10 \mu\text{m}$) and neurofilament-negative cells (Fig. 9, A and C), further supporting a glial localization. In sections from DMI-treated animals, a robust increase in FGF2-IR was observed mainly in layer V throughout the cortex in nuclei of neurofilament-positive cells (Fig. 9B and D). In addition, these cells showed a typical neuronal morphology characterized by a large cell body ($\sim 20 \mu\text{m}$) and

long processes, further suggesting that DMI increases FGF2-IR in selected neurons of the cerebral cortex. MIA had a similar effect (data not shown). In the hippocampus of control rats, FGF2-IR showed a similar glial localization (Fig. 10, A, C, and E), with the exception of the CA2 layer (Fig. 10, G and I) that is enriched in neurons. In sections of DMI-treated (and FLU-treated, not shown) rats, more neurofilament-positive cells were also positive for FGF2 (Fig. 10, D, F, and J) suggesting that DMI increases FGF2-IR in neurons also in the hippocampus.

Discussion

Early studies have provided evidence that antidepressants activate the synthesis of BDNF in selected brain areas and suggested that this trophic factor could be relevant to the therapeutic action of antidepressants (Nibuya et al., 1995). In the present study, we demonstrate that three antidepressants with different pharmacological profiles also increase FGF2 mRNA and protein levels in selected brain areas. DMI and MIA induce FGF2 expression in both cortex and hippocampus, whereas FLU preferentially targets hippocampal

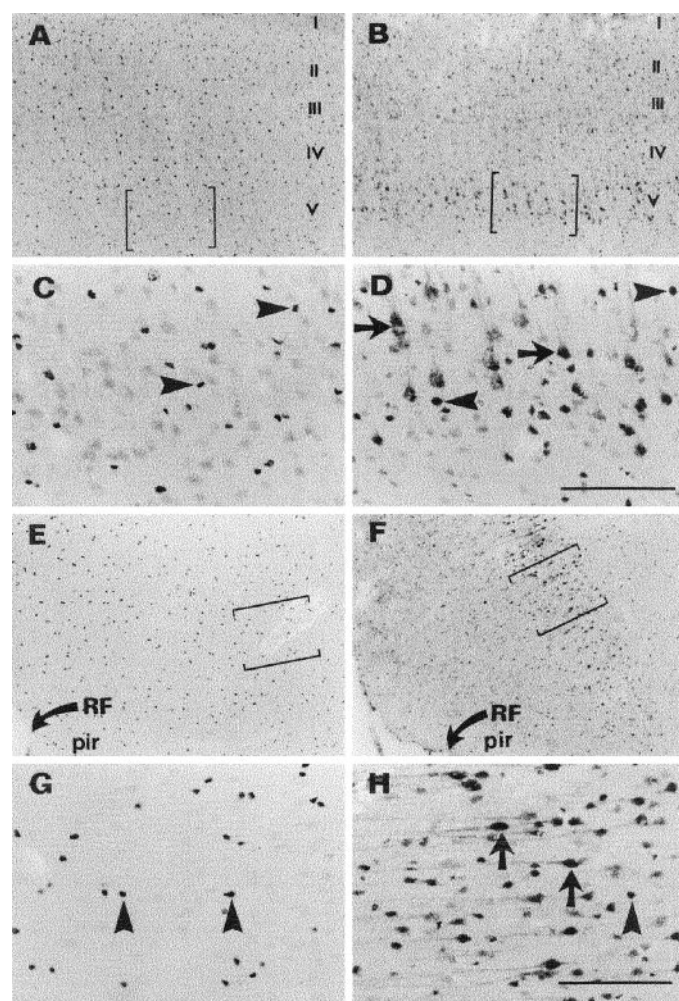


Fig. 7. DMI increases FGF2-IR in the cerebral cortex. Photomicrographs showing FGF2-IR in coronal sections of the cerebral cortex from saline- (A, C, E, and G) and DMI- (B, D, F, and H) treated rats. A & B and E & F are sections from the frontal and entorhinal cortex, respectively. C, D, G, and H are a higher magnification view of the areas in the brackets of A, B, E, and F, respectively. In control rats, FGF2-IR is mainly in small cells (arrowheads), whereas in DMI-treated rats, FGF2-IR is seen also in neurons of layer V (arrows). RF, Rhinal fissure; pir, piriform cortex. Note that FGF2-IR in neurons is in both nuclei and fibers. Scale bars, 100 μm .

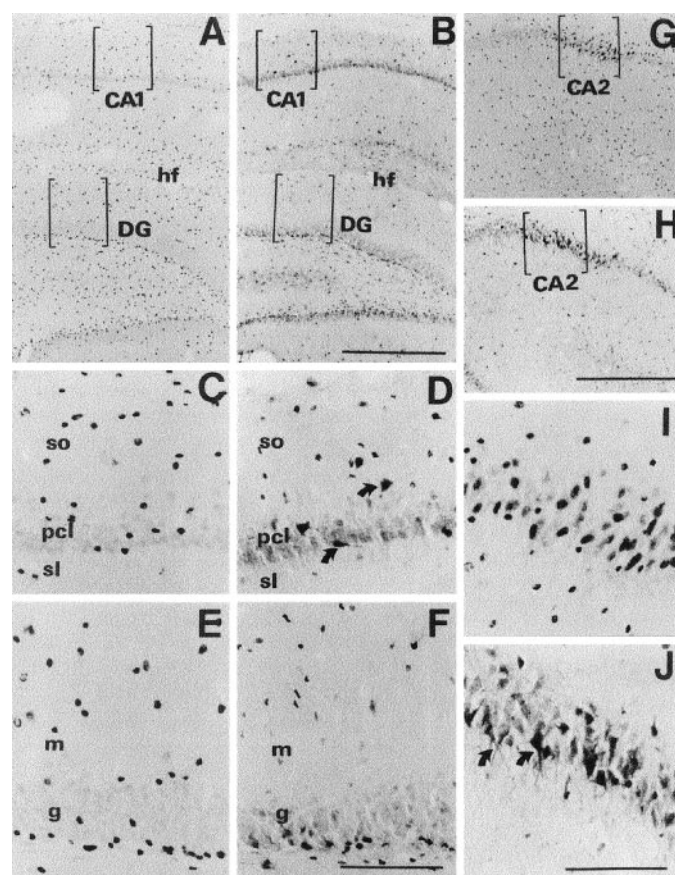


Fig. 8. DMI increases FGF2-IR in the hippocampus. Photomicrographs showing FGF2-IR in coronal sections from the hippocampus of saline- (A, C, E, G, and I) and DMI- (B, D, F, H, and J) treated rats. C, E, D, and F are a higher magnification view of the areas in the brackets of A and B, respectively. I and J are a higher magnification view of the areas in the brackets of G and H, respectively. DG, dentate gyrus; hf, hippocampal fissure; so, stratum oriens; pcl, pyramidal cell layer; sl, stratum lacunosum; m, molecular layer; g, granule cell layer. Curved arrows indicate FGF2-IR in cells morphologically similar to neurons. Scale bars in B and H, 400 μm for A, B, G, and H; scale bars in F and J, 100 μm for C, D, E, F, I, and J.

FGF2. In addition, our data have shown an increase in NGF mRNA in the entorhinal cortex by antidepressants. Therefore, we suggest a possible involvement of multiple neurotrophic factors in the mechanism of action of antidepressants.

The differential anatomical effect of these antidepressants is not surprising because each has a different mechanism of action. FLU and DMI have been considered selective serotonergic and noradrenergic uptake inhibitors, respectively, whereas MIA blocks serotonergic receptors and enhances the synaptic activity of norepinephrine (Racagni et al., 1982). Therefore, one can speculate that cortical FGF2 may be a selective target of proadrenergic antidepressant agents, whereas proserotonergic antidepressant agents may affect this neurotrophic factor in the hippocampus. Future studies with additional antidepressant treatments will support or disprove this hypothesis. Moreover, we found that the accumulation of FGF2-IR by antidepressants occurs predominantly in selected neuronal populations. This finding was unexpected because with the exception of selected neuronal

population of the CA1 and CA2 regions of the hippocampus, this neurotrophic factor is expressed throughout the brain mainly in glial cells (Gomez-Pinilla et al., 1992; Woodward et al., 1992; Hayes et al., 1995). In addition, glial cells seem to be the main source of FGF2-producing cells after CNS lesion or other stimuli. For instance, FGF2 is seen mainly in reactive astrocytes after cerebral cortex lesions (Gomez-Pinilla et al., 1992; Clarke et al., 2001) or spinal cord trauma (Mocchetti et al., 1996a). On the other hand, stimuli known to produce no damage, such as very brief episodes of electroshock (Follesa et al., 1994; Gall et al., 1994), activation of β_2 -adrenergic (Hayes et al., 1995) or glucocorticoid receptors (Mocchetti et al., 1996b) induce FGF2 expression also in glial cells. Thus, the ability of antidepressants to increase FGF2 in neurons may represent a unique and novel property of these pharmacologically active compounds. However, this hypothesis should be further tested using confocal microscopy to establish whether the increase in FGF2-IR may also occur in glial cells. In fact, we cannot completely rule out that antidepressants may increase FGF2-IR also in glial or other cells.

FGF2 is produced naturally in several forms that originate

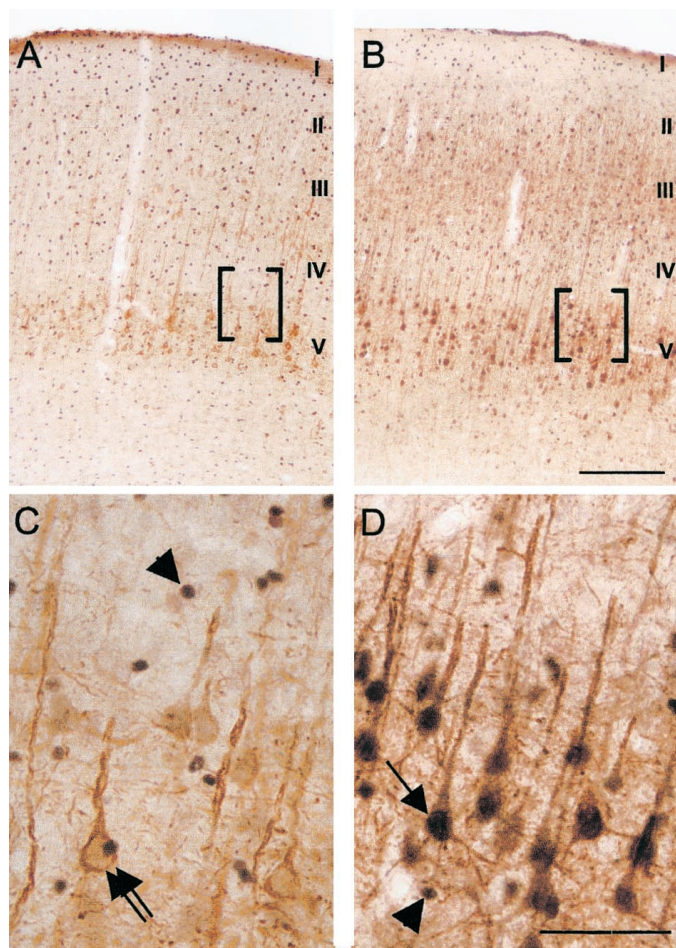


Fig. 9. Photomicrographs of double immunostaining for FGF2 and neurofilament. Sections from the cerebral cortex of control (A and C) and DMI-treated rats (B and D) were incubated with FGF2 antibody followed by neurofilament antibody. The reaction of the first antigen (FGF2) yields a black color, whereas that of the second antigen (neurofilament) is brown. C and D are a higher magnification of brackets in A and B. Arrowheads in C and D point at examples of neurofilament-negative but FGF2-positive cells; arrow in D points at an example of a neurofilament-positive cell expressing FGF2; double arrows in C indicate a typical neurofilament-positive FGF2-negative cell adjacent to a FGF2-positive glial cell. Scale bar in B, 400 μ m for A and B; in D, 100 μ m for C and D.

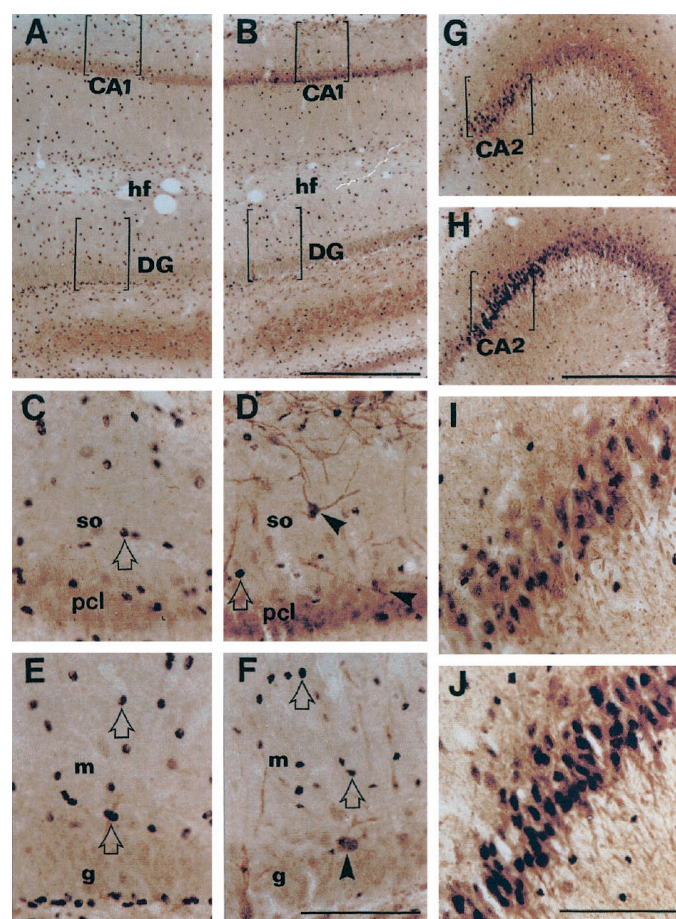


Fig. 10. DMI increases FGF2-IR in hippocampal neurons. Sections from the hippocampus of control (A, C, E, G, and I) and DMI-treated rats (B, D, F, H, and J) were incubated with FGF2 antibody and neurofilament. C, E, D, and F are a higher magnification of brackets in A and B, respectively. Control rats showed FGF2-IR mainly in neurofilament-negative cells (open arrow in C and E), whereas more neurofilament/FGF2-positive cells can be seen in DMI-treated rats (arrowheads in D and F). In the CA2 region, the intensity of FGF2-IR is increased in DMI-treated rats (H and J). Scale bar in B and H, 400 μ m for A, B, G, and H; scale bar in F and J, 100 μ m for C, D, E, F, I, and J.

from alternative splicing translation initiation sites within a single mRNA species (Florkiewicz and Sommer, 1989). The smaller translocation product has a molecular mass of 18 kDa; this isoform has a cytoplasmic localization, can be released from cells, and acts through plasma membrane receptors. The other HMM products are amino terminal extended forms of FGF2 that migrate with an apparent molecular mass of 21 and 22 kDa (Delrieu, 2000). These HMM isoforms also possess neurotrophic activity (Grothe et al., 2000) and have several characteristics: containing nuclear translocation signals; seeming to mostly localize in the nuclear compartment of cells that synthesize FGF2 (Renko et al., 1990; Bugler et al., 1991; Woodward et al., 1992); and acting through an intracellular pathways independent of cell surface receptors (reviewed in Delrieu, 2000). The results obtained by Western blot analysis have shown that the most abundant forms of FGF2 in the hippocampus and cortex are the 21- and 22-kDa isoforms suggesting that in brain tissue FGF2 is mainly nuclear. Consistent with this hypothesis, immunohistochemical evidence has shown a nuclear localization of FGF2 both in control and treated rats. However, in saline-treated rats, FGF2-IR had a nuclear localization in small cells resembling glia, whereas both DMI and FLU induced FGF2-IR in neurofilament-positive cells, suggesting that antidepressants increase FGF2 synthesis in neurons. On the other hand, antidepressant treatments increase all isoforms of FGF2; thus, these compounds might affect FGF2 release in addition to its synthesis. It remains to be established whether the induced FGF2 by antidepressants acts in an autocrine or paracrine fashion.

The induction of FGF2 evoked by antidepressants may be a common mechanism elicited by these compounds. However, the physiological and pharmacological implication of these findings is still under investigation. Depression has been associated with impaired neurotransmitter function, particularly in the noradrenergic and serotonergic systems. Moreover, loss of neuronal density has been found post mortem in brains of patients with major depression (Cotter et al., 2001; Eastwood and Harrison, 2001; Knable et al., 2001), suggesting that depression is associated with decreased or lack of trophic support. Based on these considerations, we can speculate that antidepressant agents, by restoring appropriate levels of endogenous trophic factors, may prevent loss of CNS cells or connections. Future studies aimed at examining the phenotype of FGF2 positive neurons will help to establish which cortical and hippocampal neurons may respond to the antidepressant treatment.

The induction of FGF2 protein observed in this study may explain the ability of antidepressants to increase neurogenesis (Malberg et al., 2000), presynaptic vesicles, or membrane specializations (Bal-Klara and Bird, 1990). In fact, FGF2 has been shown to induce differentiation of precursor cells from several CNS regions (Vescovi et al., 1993; Shihabuddin et al., 1997), to regulate neuronal density in the cerebral cortex during development (Raballo et al., 2000), and to be necessary for neurogenesis in the adult hippocampus (Yoshimura et al., 2001). FGF2 also facilitates propagation of human neural stem cells in vitro that, upon transplantation, have been shown to differentiate into multiple cell types (Flax et al., 1998). However, whether the neurogenic activity of antidepressant relies solely on FGF2 cannot be established at present because antidepressant agents have been shown to

increase BDNF expression and its high-affinity receptor TrkB (Nibuya et al., 1995). In addition, we observed that DMI, MIA, and FLU all increase the mRNA for another neurotrophin, NGF, in the entorhinal cortex. Therefore, it seems that antidepressants may affect neurogenesis by activating the biosynthesis of several neurotrophic factors.

The molecular mechanisms whereby antidepressants up-regulate the production of FGF2 are still under investigation. Recent evidence has shown that high levels of cAMP induce FGF2 expression in vitro (Moffett et al., 1998) and in vivo (Hayes et al., 1995). Thus, it seems that neurotransmitters or pharmacological agents that activate the protein kinase A-signaling pathway increase *FGF2* gene expression, perhaps via the activation of *cis*-regulatory elements located in the FGF2 promoter (Moffett et al., 1998). Because long-term antidepressant administrations increase nuclear levels of cAMP responsive element binding protein (Nibuya et al., 1996), it is plausible that antidepressants may affect the transcription of the *FGF2* gene by inducing the binding activity of transcription factors to *cis*-elements located in the FGF2 promoter. However, more experiments with more selective pharmacological compounds are needed to prove this hypothesis.

In conclusion, our data show a defined regional and cellular pattern of induction of FGF2 synthesis after antidepressant administration and suggest that these compounds may enhance neuronal plasticity. It remains to be established whether the pharmacologically induced FGF2 is an effective antidepressant by demonstrating the effects, if any, of the endogenous FGF2 on monoamines containing neurons in adult rats.

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Address correspondence to: Dr. Italo Mochetti, Department of Neuroscience, Georgetown University, Research Building, Room WP12, 3970 Reservoir Rd. NW, Washington DC 20007. E-mail: mocchetti@georgetown.edu