A role for nuclear β-catenin in SNRI antidepressant-induced hippocampal cell proliferation

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Increasing evidences have been accumulated during recent years suggesting a role for antidepressant drugs (ADs) as hippocampal neurogenesis enhancers, but the information about the transductional mechanisms involved in this response is very limited. We have studied in the adult rat hippocampus the effects of chronic treatment with the dual reuptake inhibitor (SNRI) venlafaxine on both cellular proliferation rate and expression of key effectors of several signaling pathways. Increased cell proliferation (BrdU incorporation) in subgranular zone (SGZ) was achieved after chronic treatment with a high dose (40 mg/kg/day) of venlafaxine. However, significant increases in the immunoreactivity of hippocampal β-catenin in SGZ were already detected after administration of a lower dose of the drug (10 mg/kg/day). Western blot and immunoelectron microscopy studies demonstrated an increased presence of β-catenin at the nuclear level. An increase in cytosolic AKT levels was also observed in venlafaxine-treated animals. These results suggest that the hippocampal proliferative effect of chronic venlafaxine, only evident when both serotonin (5-HT) and noradrenaline/norepinephrine (NE) reuptake systems are inhibited, requires a strong activation of intracellular signaling through Wnt (β-catenin translocation) and AKT/PKB pathways. This activation would probably result in an increase of the expression of cell cycle regulator genes. Furthermore pERK2/ERK2 rate was also increased in the hippocampus of AD-treated animals, while no differences in the levels of CREB and p-CREB were observed. These results illustrate the complexity of the intracellular events underlying the neurogenetic responses of ADs. They also support the relevance of such effects for the therapeutic effects of these drugs.

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1. Introduction

The most common first step in the therapeutic action of antidepressant drugs (ADs) is the increase in the monoamines serotonin (5-HT; 5-hydroxytryptamine) and/or norepinephrine (NE) extracellular levels (Richelson, 1991). This immediate effect contrasts with the onset of appreciable clinical improvement, which requires several weeks of AD treatment. Trying to clarify this apparent mismatch, a large number of studies about the trophic effects of ADs and electroconvulsive therapy (ECT) have been reported in the recent years (Altar et al., 2003, 2004; Russo-Neustadt et al., 2004). In this sense, it is well documented that chronic AD treatment enhances cell proliferation in adult rodent and nonhuman primate subgranular zone (SGZ) of hippocampus and that the time required for the differentiation and maturation of newborn neurons correlates well with the appearance of clinical response to the AD treatment (Malberg et al., 2000; Perera et al., 2007). Furthermore, the existence of a decrease in the hippocampal volume, restored after AD treatment (Vermotten et al., 2003), has been reported in depressed patients by some, but not all authors (Sheline et al., 1996; Vythilingam et al., 2004). However, the molecular pathways that control these processes are still unclear. Transduction pathways promoting cell proliferation are the main targets for studying the possible mediators for the effect of these drugs in neurogenesis. Thus, the cAMP cascade has been suggested to be involved in the neuroproliferative responses induced by ADs but contradictory results have been reported on this issue (Nibuya et al., 1996; Manier et al., 2002). In a similar way, the role of MAPK pathway is under discussion (Tiraboschi et al., 2004; Fumagalli et al., 2005) and no clear data are available with respect to AKT/PKB signaling pathway. An emerging candidate to play a key role in these processes is the Wnt pathway. Activation of the canonical Wnt pathway leads to the inhibition of GSK-3, allowing β-catenin to be stabilized in the cytosol and translocated to the nucleus, where it activates transcription of target genes (Logan and Nusse, 2004). It has been recently shown that this signaling pathway, a main
The degree of stimulation of cell proliferation varies with the type of AD used (Malberg et al., 2000). The dual reuptake inhibitor (5-HT and NE, SNRI) venlafaxine blocks both reuptake processes, with a higher potency for the 5-HT component (Béique et al., 1998) so it is possible to selectively act on the 5-HT transport or to affect both aminergic systems, depending on the doses assayed for this AD. The goal of this study has been to evaluate, in the adult rat hippocampus, the effects of chronic treatment with venlafaxine at two different doses on both the hippocampal cellular proliferation and the expression and cellular distribution of β-catenin, as well as of other key effectors of several main signaling pathways (CAMP, MAPK and PKB/akt).

2. Material and methods

2.1. Animals

Adult male albino Wistar rats (Harlan, Barcelona, Spain) weighing 175–200 g were used for this study. The animals were housed at 22 °C with a 12:12 light–dark cycle. Food and water were provided ad libitum. All experimental procedures were done according to the Spanish legislation and the European Communities Council Directive on “Protection of Animals Used in Experimental and Other Scientific Purposes” (86/609/EEC).

2.2. Antidepressant treatment and BrdU administration

Rats were implanted subcutaneously with an osmotic minipump Alzet 2002 (Alza Corp., Palo Alto, CA) which delivered 0.5 μl/h during 14 days. Sixty-six animals were used in the study: 22 rats per group were treated either with saline, 10 mg/kg/day venlafaxine, or 40 mg/kg/day venlafaxine. These doses were chosen in order to carry out a differential analysis of the effects of the drug on 5-HT and NE transport: it has been shown that low doses (10 mg/kg) selectively act on 5-HT uptake, while high doses (30–50 mg/kg) are required to achieve mixed 5-HT and NE-dependent responses in microdialysis and other functional studies (Béique et al., 1998; Felton et al., 2000; Berrocoso and Mico, 2007). Venlafaxine HCl was extracted from Vondraf 75 (Wyeth-Farma, S.A., Madrid, Spain) according to standard procedures, and easily dissolved in saline. Minipumps were removed on the 14th day of treatment.

For immunohistochemical analysis of cell proliferation, animals received 5-bromo-2′-deoxyuridine (BrdU; 4–75 mg/kg every 2 h, i.p.; Sigma, Madrid, Spain) in sterile 0.9% NaCl solution on the last day of antidepressant treatment.

2.3. Immunohistochemistry

Twenty-four hours after the last BrdU injection, rats were deeply anaesthetized with sodium pentobarbital (100 mg/kg, i.p.) and transcardially perfused with saline followed by 4% cold paraformaldehyde in PBS. Brains were removed, postfixed overnight at 4 °C and transferred to 30% sucrose in PBS at 4 °C. Serial coronal sections (45 μm) of the brains were obtained through the entire hippocampus on a cryostat and stored at −20 °C in cryoprotectant solution (25% glycerol, 25% ethylene glycol in PBS, pH 7.4).

BrdU immunohistochemistry was carried out following a previously described protocol (Malberg et al., 2000). Free-floating sections from animals from the three experimental groups (n = 9 per group) were incubated for 2 h in 50% formamide/2× SSC at 65 °C, followed by incubation in 2 N HCl for 30 min. Then sections were incubated for 10 min in 0.1 M Mborate buffer. After washing in PBS, sections were incubated in 1% H2O2 for 30 min to remove endogenous peroxidases. After several rinses in PBS, sections were incubated in PBS/0.2% Triton X-100/5% goat serum (PBS–TS) for 30 min and then incubated with monoclonal mouse anti-BrdU (1:1000; Roche Diagnostics, Barcelona, Spain) overnight at 4 °C. After several rinses in PBS–TS, sections were incubated for 2 h with biotinylated horse anti-mouse IgG secondary antibody (4 μg/ml; Vector Laboratories, Burlingame, CA), followed by amplification with avidin–biotin complex (Vector Laboratories). BrdU-positive (BrdU+) cells were labeled using DAB as chromogen (Vector Laboratories).

As BrdU+ cells are comparatively rare in the SGZ of the dentate gyrus, no counting frames were used; therefore, to quantify the number of BrdU+ cells, a modified unbiased stereology protocol using the optical fractionator method was used (West et al., 1991; Eisch et al., 2000; Malberg et al., 2000). Every sixth section throughout the hippocampus was processed and mounted on coded slides. BrdU+ cells in the SGZ of the dentate gyrus were counted by an experimenter blinded to the study code under a light microscope (Carl Zeiss Axioskop 2 Plus) at 400× magnification, excluding those that were in sharp focus in the uppermost focal plane or more than two cells away from the SGZ (optical dissecting principle). Sections (10–12) from every brain were used and an average of 660 ± 183 (mean ± st dev) cells per animal were actually counted. The total number of BrdU+ cells per section was determined and multiplied by six to obtain the total number of BrdU+ cells per hippocampus. Data were analyzed using ANOVA and Student–Newman–Keuls post hoc test using GraphPad Prism (GraphPad Software, Inc. San Diego, CA). Statistical significance was set at p < 0.05.

β-Catenin immunohistochemistry was carried out in adjacent sections of those used in the BrdU labeling studies (n = 9). After several rinses in PBS, sections were boiled in microwaves in 10 mM citric acid, pH 6.0 for 10 min. Following several rinses in PBS, sections were incubated in methanol and 3% H2O2. Then sections were transferred to blocking buffer (PBS–TS) for 30 min and incubated for 2 days at 4 °C with the following antibodies: BD Biosciences, Mountain View, CA; with β-catenin (1:500). Anti-β-catenin (BD Biosciences) in BrdU–TS, sections were incubated with biotinylated horse anti-mouse IgG secondary antibody followed by peroxidase detection with DAB or HRP conjugated donkey anti-mouse IgG secondary antibody (1:250; Jackson Immunoresearch Laboratories, Inc., West Grove, PA), followed by propidium iodide (Sigma) counterstain. Double-stained samples were analyzed with a laser confocal microscope (Zeiss LSM 510). All positive cells present in three sections from every animal were scanned for colocalization and then pseudocolor images were generated.

2.4. Subcellular fractionation and Western blot

Animals from each experimental group (n = 5) were killed by decapitation, their brains being removed from skulls and hippocampi dissected and rapidly frozen at −80 °C. Every sample was thawed and homogenized (1:15, 500–600 m) with a Potter homogenizer provided with a loosely fitting Teflon pestle in homogenization buffer (10 mM Hepes–HCl, pH 7.9, 1.5 mM MgCl2, 10 mM KCl) containing the following protease and phosphatase inhibitors: 1 mM PMSF; 10 μg/ml aprotinin; 10 μg/ml leupeptin; 10 μg/ml pepstatin A; 10 μg/ml antipain; 10 μg/ml chymostatin; 5 μg/ml trypsin inhibitor; 1 mM NaV; 1 mM NaF; 1 mM cantharidin; and 10 μM E-64. After homogenization, 250 μl of homogenate were lysated in lysis buffer (homogenization buffer containing 1% Igepal; 0.1% sodium deoxycholate, 0.2% SDS, and 0.1% Triton X-100) 30 min on ice for the total cell lysate (TCL). The remaining homogenate (250–300 μl) was processed for subcellular fractionation. Homogenate was centrifuged at 1000 g for 10 min at 4 °C, and the resulting supernatant (S1) and pellet were (P1) separated. The S1 was ultra-centrifuged at 100 000 g for 15 min at 4 °C, resulting in a supernatant (S2) cytosolic fraction, that was lysated and a pellet F2 membrane fraction, resuspended in buffer containing detergents and protease and phosphatase inhibitors and centrifuged for 10 min at 14 000 g. Nuclear proteins were isolated by high salt extraction from P1 fraction. P1 fraction was homogenized in 20 mM Hepes pH 7.9, 0.45 M NaCl, 1 mM EDTA containing protease and phosphatase inhibitors and incubated in ice for 30 min. Nuclearized proteins were recovered in the supernatant after centrifugation at 14 000 g for 10 min at 4 °C. Protein quantification was performed according to the Lowry method (Lowry et al., 1951).

Thirty-five micrograms of protein were resolved on 12.5% SDS–PAGE and transferred to PVDF (non-phosphorylated proteins) or to nitrocellulose (phosphorylated proteins) membranes. Membranes were incubated in blocking solution in PBS–TS containing protease and phosphatase inhibitors: mouse anti-β-catenin (1:1000), mouse anti-GAPDH (1:2000), mouse anti-histone H1 (1:200), mouse anti-AKT1 (1:1000) and rabbit anti-ERK1/2 (1:2000), from Santa Cruz Biotechnology, Inc. Heidelberg, Germany; mouse anti-PKCα/δ (1:10 000) and rabbit anti-actin (1:2000) from Sigma; rabbit anti-CREB (1:1000) and rabbit anti-p-CREB (1:1000), from Upstate, Charlotte, VA. After extensive washings in TBS-T (TBS/0.05% Tween 20), membranes were incubated with horse- radish peroxidase conjugated secondary antibodies. Secondary antibodies were detected with ECL Advance kit (GE Healthcare Europe GmbH, Munich, Germany). Anti-GAPDH (cytosolic marker) and anti-histone H1 (nuclear marker) antibodies were used to discard subcellular fraction contaminations. Blot quantitations were
performed by densitometric scanning using Scion Image Software. The densitometry values were normalized with respect to the values obtained with anti-actin antibody. Immunoblot data shown for each group in text and figures are expressed as mean ± standard error of the values corresponding to five animals. Samples from each animal were analyzed at least in two independent experiments. Statistical analysis was performed using ANOVA followed by Student–Newman–Keuls post hoc test.

2.5. Immunogold electron microscopy

Vibratome brain sections containing dentate gyrus (400 μm) were obtained from transcardially perfused (4% paraformaldehyde) animals from the three experimental groups (n = 3). The tissue samples were then washed with 0.1 M cacodylate buffer, dehydrated in increasing concentrations of methanol at −20 °C, embedded in Lowicryl K4M at −20 °C and polymerized under ultraviolet irradiation. Ultrathin sections were mounted on nickel grids and sequentially incubated with 0.1 M glycine in PBS for 15 min, 5% BSA in PBS for 30 min and mouse anti-β-catenin (1:50) antibody (diluted in 50 mM Tris–HCl, pH 7.6, containing 1% BSA and 0.1 M glycine) for 1 h at 37 °C. After washing, the sections were incubated with goat anti-mouse antibody coupled to 15 nm gold particles (British BioCell International, Cardiff, UK; 1:50 in PBS containing 1% BSA). After immunogold labeling, the grids were stained with lead citrate and uranyl acetate and examined with a Philips EM208 electron microscope operated at 60 kV. Images were digitized and then analyzed using ImageJ software (http://rsb.info.nih.gov/ij/). Statistical analysis of gold particles densities was performed using ANOVA followed by Student–Newman–Keuls post hoc test.

3. Results

3.1. Effect of venlafaxine on cell proliferation

The analysis of the number of BrdU+ cells showed that chronic (14 days) venlafaxine treatment produces a significant increase in cell proliferation in SGZ of dentate gyrus, only when a high dose (40 mg/kg/day) of this AD was administered (4888 ± 266 BrdU+ cells; 44.8 ± 8.4% increase vs saline group, 3374 ± 355 BrdU+ cells; p < 0.01; Fig. 1). No statistically significant changes in cell proliferation rate were observed in the animals receiving 10 mg/kg/day of venlafaxine (3621 ± 335 BrdU+ cells; 7.3 ± 10.6% increase vs saline group). Furthermore, significant differences in the cell proliferation rates were observed when comparing 10 and 40 mg/kg/day venlafaxine dose groups (34.9 ± 7.9% increase vs 10 mg/kg/day; p < 0.05). All data showed homogeneity of variance (Bartlett’s test, p = 0.747).

3.2. Modifications in the expression patterns of main effector proteins of signal transduction pathways after venlafaxine treatment

Measurement of β-catenin expression in hippocampal total homogenates revealed significantly higher levels of this protein after chronic treatment with either doses of venlafaxine compared with saline treated rats (73 ± 13%, p < 0.05 and 175 ± 36%, p < 0.001 increases for 10 and 40 mg/kg/day doses, respectively; Fig. 2a and b). After cellular fractionation, β-catenin was detected in the membrane-associated and nuclear fractions. In both of them, significant increases in the levels of this protein were found after chronic treatment at either doses of venlafaxine when compared with saline rats, these increases being more pronounced in the nuclear fraction. Chronic treatment with 40 mg/kg/day resulted in amounts of this protein higher than those observed for 10 mg/kg/day in the two cellular fractions studied (62 ± 25%, p < 0.05 in membrane-associated fraction, 88 ± 9%, p < 0.001 in nuclear fraction).

Detectable AKT protein levels were obtained in the cytosolic and nuclear fractions, as well as in the TCL (Fig. 2b and c). A dose-dependent increase in the levels of expression of this protein was observed in the total homogenate (28 ± 11%, p = n.s.; 84 ± 31%, p < 0.05, for 10 and 40 mg/kg/day doses, respectively). In the

![Fig. 1. Chronic venlafaxine treatment increases the number of BrdU+ cells in the SGZ of adult rat hippocampus when administered at a high dose. BrdU+ cells were labeled using DAB as a chromogen. Representative microphotographs of BrdU immunohistochemistry from saline (A), 10 mg/kg/day venlafaxine (B), and 40 mg/kg/day venlafaxine (C) chronically treated animals. Cresyl violet counter-staining. (D) The number of BrdU+ cells in the subgranular zone (SGZ) is significantly increased after chronic treatment with 40 mg/kg/day venlafaxine. The results are the mean ± standard error number of BrdU+ cells in SGZ. See Section 2 for further details; **p < 0.01; *p < 0.05. GCL, granule cell layer; H, hilus; bar = 100 μm.](image-url)
cytosolic subfraction, increased levels of AKT were found in tissues from animals chronically treated at either venlafaxine doses (47 ± 13%, p < 0.05 and 69 ± 23%, p < 0.05). In contrast, a significant dose-dependent decrease in the amount of AKT was detected in the nuclear fraction (−22 ± 6%, p < 0.05 and −38 ± 8%, p < 0.01).

No significant changes in the levels of expression of both CREB and p-CREB were observed in the nuclear fractions of animals chronically treated with either dose venlafaxine when compared with saline group. Phosphorylated CREB/CREB ratio was not affected either (not shown).

Immunoblot analysis of the expression of the two isoforms of ERK protein (ERK1 and ERK2) in total homogenates (Fig. 3) showed no change after chronic treatment with 10 or 40 mg/kg/day venlafaxine with respect to saline administered rats. On the other hand, increased levels of the phosphorylated form (activated form) of both isoforms of this protein were observed in TCL after the AD treatment (96 ± 8%, p < 0.05 and 141 ± 49%, p < 0.01 for pERK1 for 10 and 40 mg/kg/day, respectively. 80 ± 12%, p < 0.05 and 101 ± 38%, p < 0.05 for pERK2 for 10 and 40 mg/kg/day, respectively.). In this sense, pERK1/ERK1 and pERK2/ERK2 ratios showed at least a 1.5-fold change after venlafaxine chronic treatment.

3.3. Increased immunoreactivity of β-catenin in the SGZ after chronic treatment with venlafaxine

β-Catenin staining throughout the hippocampus presented a distribution pattern characterized by a weak immunostaining over the GCL and positive immunostained clusters detected in the SGZ of the dentate gyrus. Saline group presented levels of immunostaining under 25% with respect to the highest immunoreactivity found. Animals treated with 10 mg/kg/day venlafaxine showed a mean number of immunopositive clusters per section ranging 50–75% with respect to the highest immunoreactivity found. Mean number of immunopositive clusters for β-catenin in the SGZ of animals treated with 40 mg/kg/day venlafaxine was over 75% with respect to the highest immunoreactivity found (Table 1 and Fig. 4).

The clusters of β-catenin immunolabeling observed in the SGZ were studied in order to detect nuclear β-catenin presence. No presence of this protein could be detected in nucleus from SGZ cells when they were observed under confocal microscopy (Fig. 5) in any sample from animals from the three experimental groups. GCL cells’ nuclei neither were β-catenin positive when examined.

No nuclear BrdU+/β-catenin− colocalization could be observed in any sample analyzed, but all BrdU+ nuclei observed showed high β-catenin labeling surrounding them and were embedded in β-catenin clusters (Fig. 5).

3.4. Detection of nuclear β-catenin in SGZ cells by immunoelectron microscopy

Presence of β-catenin in nuclei from SGZ cells could be observed by immunoelectron microscopy. In the cells where this detection was carried out, the immunolabeled nuclei presented the euchromatin configuration typical of neuronal populations (Peters et al., 1991). The presence and distribution of β-catenin appeared in DNA euchromatinic regions, with no presence in nucleoli or heterochromatin, compatible with a transcription factor distribution pattern. The low density of gold particles observed in nucleus from SGZ and CGL cells from animals of saline group strongly contrasts with those observed after chronic treatment with venlafaxine (Fig. 6) showing an increased number of gold particles per μm² (p < 0.05) as well as tendency to the decrease in the average distance between particles (data not shown).
4. Discussion

This study demonstrates for the first time that chronic treatment with the SNRI venlafaxine increases both the hippocampal cell proliferation and the translocation rate to the nucleus of β-catenin, a main effector of the Wnt pathway.

The positive effects reported in the last years for ADs and ECT on adult hippocampal cell proliferation (Malberg et al., 2000; Madsen et al., 2000; Czéh et al., 2001; Sairanen et al., 2005; Dranovsky and Hen, 2006) support a new hypothesis about “the mechanisms through which ADs exert their therapeutic actions. Fluoxetine has been the AD more widely used in these studies (Malberg et al., 2000; Sairanen et al., 2005; Santarelli et al., 2003). However, it is being suggested that the use of dual reuptake inhibitors, such as venlafaxine, could be of special interest in the treatment of some major depression cases, under certain clinical conditions (Tzanakaki et al., 2000; Stahl et al., 2002; Poirier and Boyer, 1999; Mehtonen et al., 2000). We have studied the state of cell proliferation after 14 days of treatment with venlafaxine, using two doses: 10 mg/kg/day, which mainly acts on 5-HT transporter, and 40 mg/kg/day, a dose that inhibits the reuptake process through both 5-HT and NE transporters (Béique et al., 1998). A significant increase in cell proliferation in SGZ was observed in the animals administered with 40 mg/kg/day, while no effect was found following the administration of 10 mg/kg/day. These results are in contrast with previous data (Khawaja et al., 2004), which showed augmentation of cell proliferation in rats treated for 14 days with 10 mg/kg/day venlafaxine. Two methodological differences in the experimental design could explain this discrepancy. First, in Khawaja et al.’s (2004) study, venlafaxine was administered i.p. while we used s.c. route (osmotic minipumps). Second, in the mentioned study BrdU was injected along four consecutive days following the last administration of venlafaxine, while in this work the cell proliferation analysis was carried out 24 h after removing the osmotic minipump. These results suggest that, depending on several factors (type of drug, route of administration and duration), the activation of the serotonergic system might be not enough to elicit by itself a proliferative response, thus requiring the concomitant inhibition of NE transport. Furthermore, they are in good agreement with the reported ability of venlafaxine to prevent the decrease in hippocampal cell proliferation induced by chronic stress (Xu et al., 2006).

These results strongly support that ADs are potential enhancers of hippocampal cell proliferation. In order to address the signaling pathways involved, we studied the effects of venlafaxine on the amount and cellular distribution of main effector proteins of several signaling pathways involved in cell proliferation. β-Catenin expression was increased in both TCL and purified nuclear fraction.
in venlafaxine-treated animals in a dose-dependent manner, suggesting an increase in translocation of β-catenin to cell nucleus. This finding correlates well with that reported in a previous study using ECT (Madsen et al., 2003). The fact that BrdU+ cells were embedded in immunopositive β-catenin clusters further supports the relevance of this protein in hippocampal cell proliferation. Interestingly, GSK-3 inhibitors, which allow stabilization of β-catenin, have been reported to exert antidepressant-like effects in animals (Kaidanovich-Beilin et al., 2004; Gould et al., 2006). The role of β-catenin in affecting gene expression would be mediated by the interaction with TCF/LEF DNA-binding proteins (Logan and Nusse, 2004; van de Wetering et al., 1997). Target genes would include, in addition to cell cycle regulator genes, myc and cyclinD1 (He et al., 1998; Shtutman et al., 1999), Wnt signaling genes, thus leading to a fine feedback regulation of this pathway (Logan and Nusse, 2004). Our study was not aimed to directly analyze the induction of neurogenesis by chronic venlafaxine. However, taking into account that the adult hippocampal stem/progenitor cells (AHPs) express...
receptors for Wnt proteins (Madsen et al., 2003), it could be suggested that chronic venlafaxine would result in a double β-catenin-dependent activation of AHPs: a direct activation of neural precursors in SGZ to re-enter in cell cycle, and an indirect regulation of surrounding differentiated cells, such as astrocytes, in order to secrete Wnt proteins, that would act on hippocampal progenitor cells, leading to a further increase in neurogenesis (Lie et al., 2005). Activated AHP cells are able to divide through mechanisms that involve the expression of CyclinD genes, producing daughter cells that can differentiate to neurons (neurogenesis) or astrocytes (gliogenesis) (Fig. 7). In this regard, it has been recently shown that chronic treatment with antidepressants induces an increase of symmetric divisions of an early progenitor cell class in the dentate gyrus (Encinas et al., 2006).

We could not detect any clear increase in cell proliferation after treatment with the lower dose of venlafaxine (10 mg/kg/day), which also induced the increase in β-catenin. This suggests that a high level of induction of the Wnt pathway is required to affect cell proliferation. Alternatively, these results could indicate the involvement of other signaling pathways in this response. No information about hippocampal β-catenin expression in tissues from depressed patients is available, although no changes have been found in cortical samples (Beasley et al., 2002).

We tried to detect the presence of nuclear β-catenin in SGZ and GCL cells, as well as in BrdU+ nuclei from SGZ, by immunofluorescence microscopy techniques. A clear perinuclear β-catenin staining was detected surrounding BrdU+ nuclei, but no nuclear staining was demonstrated as previously reported in the literature (Madsen et al., 2003; Lucas et al., 1999) where the use of immunoelectron microscopy has been suggested to overcome this limitation (Lucas et al., 1999, 2001); our immunoelectron microscopy

Fig. 6. Electron microscopy photomicrographs showing nuclei from granule cells from saline (A), 10 mg/kg/day venlafaxine (B), and 40 mg/kg/day venlafaxine (C) chronically treated animals. Density of gold particles in the nucleus of granule cells of the hippocampus of rats from the three experimental groups (D). Inset (C) shows a higher magnification photomicrograph of the area in the black box. Data are expressed as mean ± standard error number of gold particles per μm². β-Catenin positive labeling (arrows) is increased in venlafaxine-treated animals. Immunogold labeling stained with lead citrate and uranyl acetate. See Section 2 for further details. Nu: nucleolus; *p < 0.05; bar = 0.2 μm; gold particles’ diameter = 15 nm.

Fig. 7. Diagram depicting the suggested effects of AD on Wnt pathway in the SGZ. Adult hippocampal stem/progenitor cells (AHPs), neurons and glial cells are present in SGZ and adjacent GCL. AD treatment increases the extracellular levels of 5-HT and NE, which, in neurons and glial cells, through serotonin and adrenergic receptors, elicit a number of reactions that lead to AKT activation. Inhibition of GSK-3 by AKT increases β-catenin cytoplasmatic levels and promotes the translocation to the nucleus of this protein. In these very differentiated cells, no expression of cell cycle induction proteins will take place. However, they express Wnt pathway proteins, among them Wnt receptors agonists, which are secreted to the extracellular space. There, secreted Wnt agonists act on Frizzled receptors (FZr) on surrounding cells, neurons and glia, where they enhance the activation of the Wnt pathway, and AHPs, where they elicit the expression of cell cycle related proteins, as CyclinD, promoting cell division.
studies focused on nuclei presenting the neuronal euchromatin pattern, and confirmed the presence of β-catenin in the nucleus of SGZ cells in all experimental groups. The pattern of distribution found, in euchromatin and no nucleolar presence, confirms the role of this protein in the regulation of target genes’ expression. In venlafaxine-treated animals, nuclear β-catenin density was higher than in the saline group, and gold particles usually appeared forming clusters of several units. This is, to our knowledge, the first report of an increased level of nuclear β-catenin following AD treatment.

Taking into account that β-catenin is also a component of the cadherin/catenin complex, stabilizing the actin cytoskeleton and mediating cell adhesion (Cadigan and Nusse, 1997), the increased levels of β-catenin detected after venlafaxine chronic treatment in the membrane-associated fraction could suggest a positive effect in stabilizing synapses and increasing dendritic arborization. This agrees with recent reports demonstrating, first, that the intracellular level of cadherin/catenin complex is a limiting factor in dendritic morphogenesis (Yu and Malenka, 2003) and, second, that AD treatment increases the formation of dendritic spines in rat hippocampus (Hajszan et al., 2005).

The AKT/PKB signaling is strongly involved in the regulation of several cell responses, such as cell proliferation, growth and cell survival (Brazil and Hemmings, 2001). Interestingly, the existence of a significantly lower activity of this protein in occipital cortex of depressed suicide subjects has been recently reported (Hsiung et al., 2003). Our results regarding AKT levels after venlafaxine treatment (increase in cytosolic and decrease in nuclear fractions) suggest that this protein is being accumulated in the cytosol, in order to be subsequently activated in the cell membrane. Since GSK-3 is directly inhibited by AKT (Cross et al., 1995), this recruitment of cytosolic AKT would lead to a strong inhibition of this kinase, resulting in further stabilization of β-catenin.

Our results show that CREB levels were not affected by chronic venlafaxine treatment. A tendency to the decrease in cortical CREB levels from depressed patients has been reported (Dowlatshahi et al., 1998; Yamada et al., 2003), although no study has been performed in hippocampus. In line with that, several reports indicate that AD treatment could increase CREB function, although the level of response appears to depend on several factors (Tiraboschi et al., 2004; Frechilla et al., 1998; Thome et al., 2000). Fluoxetine has been reported to be more effective in increasing the expression and phosphorylation of hippocampal CREB, when compared to ADs affecting NE uptake (reboxetine and desipramine) (Tiraboschi et al., 2004; Frechilla et al., 1998). Furthermore, a reduction in the ability to phosphorylate CREB in human neuroblasts has been described following reboxetine and desipramine administration, but not after venlafaxine treatment (Manier et al., 2002). These results illustrate the variability of the modifications induced by ADs on CREB expression, which strongly depends on the particular drug used.

Few data are available about the effect of ADs on the components of MAPK pathway (Tiraboschi et al., 2004; Fumagalli et al., 2005). Our study agrees with most previously published results, showing no consistent change in the levels of ERK1/2 in hippocampus after venlafaxine treatment, supporting that their expression is not affected by ADs. Regarding PErK1 and PErRK2, data in the literature are contradictory: while no changes have been reported after treatment with imipramine, decreases (nuclear PErK1/2), increases (nuclear PErK1) and lack of changes have been observed as a consequence of fluoxetine treatment (Tiraboschi et al., 2004; Fumagalli et al., 2005). Although caution is needed when analyzing these results, our data support the activation of the MAPK pathway following chronic administration with venlafaxine, and are in agreement with results from postmortem studies showing a decrease in brain pERK1/2 levels in depressed patients (Hsiung et al., 2003).

In conclusion, our results indicate that chronic treatment with venlafaxine induces nuclear translocation of β-catenin, probably through activation of Wnt and AKT pathways. This effect could be mediating the increased hippocampal cell proliferation observed, thus reinforcing the role of neurotransmitter mechanisms in the therapeutic activity of ADs.

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