Antidepressant imipramine induces human astrocytes to differentiate into cells with neuronal phenotype

Stefano Cabras1, Francesca Saba1, Camilla Reali1, Maria Laura Scorciapino1, Annarita Sirigu1, Giuseppe Talani2, Giovanni Biggio2 and Valeria Sogos1

1 Department of Cytomorphology, University of Cagliari, Italy
2 Department of Experimental Biology Bernardo Loddo, Section of Neuroscience, University of Cagliari, Italy

Abstract

Several recent studies have expanded our conception of the role of astrocytes in neurogenesis, proposing that these cells may contribute to this phenomenon not only as a source of trophic substances, but also as stem cells themselves. We recently observed in vitro that human mature astrocytes can be induced to differentiate into cells with a neuronal phenotype. Antidepressant drugs have been shown to increase neurogenesis in the adult rodent hippocampus. In order to better understand the role of astroglia in antidepressant-induced neurogenesis, primary astrocyte cultures were treated with the antidepressant imipramine. Cell morphology was rapidly modified by treatment. In fact, whereas untreated astrocytes showed large, flat morphology, after a few hours of treatment cells exhibited a round-shaped cell body with long, thin processes. The expression of neuronal markers was analysed by immunocytochemistry, Western Blot and RT–PCR at different treatment times. Results showed an increase in neuronal markers such as neurofilament and neuron-specific enolase (NSE), whereas glial fibrillary acidic protein (GFAP) and nestin expression were not significantly modified by treatment. Similar results were obtained with fluoxetine and venlafaxine. Hes1 mRNA significantly increased after 2 h of treatment, suggesting involvement of this transcription factor in this process. These results confirm the role of astrocytes in neurogenesis and suggest that these cells may represent one of the targets of antidepressants.

Received 22 July 2009; Reviewed 27 August 2009; Revised 23 December 2009; Accepted 1 February 2010; First published online 31 March 2010

Key words: Adult neurogenesis, antidepressant, astrocyte, differentiation, imipramine.

Introduction

Depression is a common and devastating illness affecting a large number of individuals at some point in their lives and is treated with antidepressant drugs. The molecular and cellular mechanisms involved in depression and in the therapeutic action of antidepressants are not well understood. Most antidepressants exert their effect by inhibiting serotonin and/or noradrenaline reuptake. However, in spite of a rapid increase in extracellular levels of these monoamines, therapeutic action of these drugs usually requires several weeks or months (Wong & Licinio, 2001). These observations suggest that mechanisms other than monoaminergic activation should be involved in the clinical action of antidepressants. Monoamine depletion determines a decline in hippocampal neurogenesis, while an increase in hippocampal neurogenesis is observed following elevation of serotonin and/or norepinephrine levels (Brezun & Daszuta, 1999; Kulkarni et al. 2002). More recently, brain imaging and post-mortem studies revealed morphometric alterations in specific brain areas of depressed patients (Bremner et al. 2000; Campbell et al. 2004; MacQueen et al. 2003; Sheline et al. 1996). In addition, it has been shown that in laboratory animals, chronic stress decreases neurogenesis and the trophic properties of neurons in the adult hippocampus, two mechanisms contributing to the reduction in hippocampal volume observed in subjects with mood disorders (Gould et al. 1998; McEwen, 1999). In contrast, early, long-lasting antidepressant treatment prevents...
Adult neurogenesis occurs only in the subventricular zone of the lateral ventricles (SVZ) and in the subgranular zone of the hippocampal dentate gyrus (SGL). In these brain areas, neural stem cells continue to proliferate and give rise to new neurons throughout the lifetime of animals, as well as humans (Eriksson et al. 1998; Gage, 2000; Manganas et al. 2007). Several authors have suggested that adult neural stem cells belong to the astrocytic lineage (Goldman, 2003; Gotz et al. 2002; Steindler & Laywell, 2003).

Astrocytes are the most abundant cells in the human central nervous system and have long been seen as providing structural, metabolic and trophic support for neurons. Moreover, during development astrocytes support the proliferation, survival and maturation of neurons (Nakayama et al. 2003), and are involved in synapse formation and plasticity (Allen & Barres, 2005).

Several recent studies have expanded our conception of their role in neurogenesis and gliogenesis. In fact, it has been proposed that astrocytes may contribute to this phenomenon not only as a source of trophic substances regulating neurogenesis (Song et al. 2002), but may retain stem cell-like properties (Doetsch, 2003). Doetsch et al. (1999) demonstrated in vitro that glial fibrillary acidic protein (GFAP+) astrocytes can produce neurospheres containing multipotent progenitor cells, a phenomenon that in mice gives rise to neural stem cells in the subventricular zone. In fact, a distinct population of GFAP-expressing cells, behaving as neural stem cells, but displaying ultrastructural characteristics and other markers of astrogial cells, coexist with astrocytes in the germinatal zones of the adult brain, and possibly in non-neurogenic regions (Colak et al. 2008; Doetsch, 2003; Ihrie & Alvarez-Buylla, 2008; Platel et al. 2008).

Recently, we observed in vitro that human astrocytes treated with a cocktail of protein kinase activators and FGF-1 can differentiate into cells with a neuronal phenotype (Pillai et al. 2006). On the basis of these findings, it was of interest to determine whether antidepressant drugs acting on astrocytes may facilitate their differentiation into neurons. In the present study, primary astrocyte cultures established from human fetal brain were treated with imipramine to induce their differentiation into cells having a neuronal phenotype. Imipramine is one of the most effective antidepressant drugs in patients with severe chronic depression. Moreover, in rodents it is able to prevent the action of stress on neurogenesis and to reverse the loss of hippocampal volume (Chen et al. 2008; Li et al. 2008).

**Method**

**Tissue collection**

Fetal tissues, whose use had been approved by the Ethics Committee of the Internal Medicine Institute of the University of Cagliari, were obtained from medically induced or spontaneous abortions.

**Tissue cultures**

Human fetal brain cultures were obtained as previously described (Ennas et al. 1992; Segos et al. 1990). In brief, tissues from 8- to 12-wk-old whole fetal brains were incubated with sterile trypsin (0.2%) for 30 min at 37 °C, mechanically dissociated and diluted in Dulbecco’s Modified Eagle’s Medium (DMEM, Gibco, USA) plus 10% (v/v) fetal calf serum (FCS, Gibco). Cells were then plated in polylysine pretreated dishes and trypsinized every 2 wk. Astrocytic cultures were obtained from these cultures after 6–8 wk in vitro (Ennas et al. 1992).

**Cell treatment**

After at least four passages, cells were plated at a density of 5 × 10⁴ cells/ml in 10-cm tissue culture dishes for Western blot and reverse transcription–polymerase chain reaction (RT–PCR) and in 3.5-cm dishes containing glass coverslips for immunocytochemistry. After 24 h, cells were treated with 1 μM imipramine or fluoxetine or venlafaxine in DMEM/F12 1:1 supplemented with insulin-transferrin-sodium selenite (ITS; Sigma, USA) 1%. Drug concentration was chosen on the basis of a dose–response curve, as the lowest concentration able to induce morphological modifications in astrocytes after 24 h.

At various time intervals, cultures were fixed by immersion in 4% paraformaldehyde in PBS for 1 h, washed, and processed for immunocytochemistry or for RT–PCR and Western blot.

**Immunocytochemistry**

Cells on coverslips were fixed at −20 °C with cold methanol for 4 min. For staining, samples were rehydrated in PBS/0.2% (v/v) Triton X-100 (USB, USA) and preincubated with normal goat serum (1:5, Vector, USA) for 30 min. Cells were then overlaid for 60 min at room temperature with the following primary antibodies diluted in PBS/0.2% (v/v)
Triton: monoclonal anti-nestin (1:100, Chemicon, USA); monoclonal anti-GFAP (1:100, Cymbus Biotechnology, USA); polyclonal rabbit-anti-GFAP (1:200, Dako, USA); polyclonal chicken anti GFAP (1:200, Chemicon, USA); polyclonal anti-neurofilament 150 kDa (NF-M, 1:250, Chemicon); polyclonal anti-synaptophysin (1:50, Dako), polyclonal anti-S100 (1:50, Dako). Texas Red-conjugated anti-mouse IgG (1:250, Jackson, USA), fluorescein isothiocyanate–conjugated (FITC) anti-rabbit IgG (1:500, Molecular Probes, USA) and aminomethylcoumarin (AMCA)-conjugated (FITC) anti-rabbit IgG (1:500, Molecular Probes, USA) were used as secondary antibodies. Negative controls were routinely performed for each experiment, incubating the samples with non-immune serum and then with appropriate secondary antibody. The imaging was carried out using an Olympus BX41 fluorescence microscope.

Cell counts were obtained from three separate experiments using AnalySIS 3.1 (Soft Imaging System GmbH, Germany). Three coverslips per experiment were analysed, and 5–15 fields on the same slide were counted at 20× magnification for a total of approximately 20–60 cells/field.

In other experiments, cells were incubated for 60 min at room temperature with antibodies against neuron-specific enolase [(NSE); 1:20, monoclonal, Cymbus Biotechnology]. Subsequently, slides were rinsed three times in PBS–Triton and incubated for 30 min with biotinylated anti-mouse IgG (1:200, Vector). Samples were rinsed and incubated with avidin-D-conjugated horseradish peroxidase (1:500, Vector) followed by 10 min incubation with DAB tablet sets (Sigma Fast). Negative controls were incubated with non-immune serum and with appropriate secondary antibody.

5-bromo-2-deoxyuridine (BrdU) cell proliferation assay

Cells were incubated with 2 μM BrdU (Sigma) plus 1 μM imipramine, fixed at –20 °C with cold methanol for 4 min, rehydrated in PBS/0.2% (v/v) Triton X-100, incubated with 2 M HCl at 37 °C for 10 min and then washed with borate buffer and with PBS/0.2% Triton. Samples were then incubated with normal goat serum (1:5, Vector) for 30 min and subsequently with a monoclonal anti-BrdU antibody (1:10, Dako) for 60 min. Texas-Red-conjugated anti-mouse IgG was used as secondary antibody (1:250, Jackson). Samples were then incubated with donkey serum (1:5, Sigma) for 30 min and subsequently with a polyclonal anti-NF-M antibody (1:250, Chemicon) for 60 min, followed by FITC-conjugated anti-rabbit IgG (1:500, Molecular Probes) as secondary antibody. Negative controls were incubated with non-immune serum and with secondary antibodies.

Western blot

Cells were lysed with sodium dodecyl sulfate (SDS) 2%. Protein concentration was measured according to Lowry’s method (Lowry et al. 1951). Loading buffer [75 mM Tris–HCl (pH 6.8), 20% glycerol, 5% 2-mercaptoethanol, 0.001% Bromophenol Blue] was subsequently added and samples placed in boiling water for 3 min. Next 15–30 μg of protein was run on 10% or 8% SDS–polyacrylamide gel, depending on molecular weight. Separated proteins were electrophoretically transferred onto polyvinylidene fluoride (PVDF) membrane (Hybond-P, Amersham, USA) and blocked with 5% non-fat dry milk overnight at 4 °C. Immunodetection was performed with antibodies against nestin (1:1000, monoclonal, Chemicon), GFAP (1:5000, polyclonal, Dako), neurofilament-M (1:5000, polyclonal, Chemicon) and NSE (1:5000, monoclonal, Cymbus Biotechnology). The membrane was washed and incubated with horseradish-peroxidase-conjugated anti-mouse IgG (1:1000, Chemicon) or anti-rabbit IgG (1:5000, Chemicon). After washing, protein bands were detected with SuperSignal West Pico chemiluminescent substrate (Pierce, USA). Filters were reprobed with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (1:600, monoclonal, Chemicon) to confirm equal protein loading.

RT–PCR

Total RNA was isolated from cell cultures using Trizol reagent (Invitrogen) according to Chomczynski’s method (Chomczynski, 1993). RNA concentration was measured by a spectrophotometer at 260 nm. Identical amounts of RNA were reverse-transcribed into cDNA, subsequently amplified by polymerase chain reaction (PCR) with neural-specific primers (Table 1). To obtain quantitative PCR with a non-radioactive label, digoxigenin-11-dUTP (DIG, Roche, USA) was incorporated during PCR reaction. An aliquot of the PCR reaction mixture was electrophoresed in 2% agarose gel (Sigma) in TAE (Tris–acetate/EDTA) buffer to allow adequate separation of DNA, which was transferred to a nylon membrane by blotting for 16 h in 10× SSC. The blot was briefly washed in buffer A [0.1 M maleic acid; 0.15 M sodium chloride (pH 7.5), plus 0.3% Tween-20] and incubated for 30 min at room temperature in blocking buffer (1% blocking reagent, Roche, in buffer A). This was followed by a 30-min
incubation period with an antidigoxigenin-IgG conjugated to alkaline phosphatase (Roche) diluted in blocking buffer. The blot was washed and chemiluminescent detection performed by incubating the membrane for 5 min in a chemiluminescent substrate for phosphatase detection (CSPD, Roche). The membrane was sealed in a clear plastic bag and exposed to X-ray film (Kodak X-OMAT) for 2–10 min in an X-ray cassette at room temperature. Signals corresponding to each molecule of interest and GAPDH mRNA were scanned using a densitometer (Hoefer Scientific Instruments, USA). mRNA amounts were normalized by comparison with GAPDH levels. Data are presented as percentage of control. Differences in mRNA amounts between control and treated cells were evaluated using Student’s $t$ test for paired samples.

**Whole-cell electrophysiological recording**

Cells on coverslips were transferred to a perfusion chamber (Warner Instruments, USA), and visualized with an infrared-differential interference contrast microscope ($40 \times$). Cells were perfused with an external solution which contained 130 mm NaCl, 5 mm KCl, 2 mm CaCl$_2$, 1 mm MgCl$_2$, 10 mm Hepes–NaOH (pH 7.3) and 11 mm glucose. Whole-cell current-clamp experiments were performed with an Axopatch 200-B amplifier (Molecular devices, USA). Recording pipettes (borosilicate capillaries with a filament; outer diameter, 1.5 mm) (Sutter Instruments, USA) were prepared with a two-step vertical puller (Sutter Instruments) and had resistances between 4 and 6 MΩ. Pipette capacitance and series resistance were compensated, the latter at 60%. Signals through the patch-clamp amplifier were filtered at 2 kHz and digitized at 5.5 kHz with commercial software (pClamp 9; Axon Instruments, USA). Pipettes were filled with an internal solution containing: 135 mm K-gluconate, 10 mm MgCl$_2$, 0.1 mm CaCl$_2$, 1 mm EGTA, 10 mm Hepes (pH 7.3) and 2 mm Na$_2$-ATP.

**Small interfering RNA (siRNA)-mediated down-regulation of Hes1**

The lentiviral shRNA expression system to knockdown Hes1 expression is commercially available from...
SantaCruz Biotechnology (USA). Cultured astrocytes were infected at a multiplicity of infection of 2:1 in DMEM containing 5 μg/ml polybrene overnight. The medium was then replaced with regular culture medium. Four days after infection, the cells were selected for puromycin resistance (1 μg/ml). Two weeks after infection, the cells were treated with imipramine as described above.

**Results**

**Morphological modification**

In the present study, we used astroglial cultures from human fetal brains. This model had previously been characterized using specific markers, indicating that in our cultures only mature astrocytes are present, and endothelial cells, fibroblasts, oligodendrocytes, neurons and microglia are excluded (Ennas et al. 1992; Pillai et al. 2006; Sogos et al. 1990). However, characterization was performed for each individual experiment showing that 90–95% of cells express mature astrocytic markers, such as GFAP and S-100 (Fig. 1a, b). Cells maintained astroglial phenotype for several weeks, as already observed in similar models (Cristófol et al. 2004; Hua et al. 2002; Kyoung Pyo et al. 2004). In control conditions astrocytes (Fig. 1c) exhibit flat morphology with short processes. Treatment with the antidepressant imipramine (1 μM) induced rapid morphological modification. In fact, 8 h after drug exposure (Fig. 1d), some cells exhibited a round cell body with extending processes (arrows). The percentage of cells modifying their morphology increased progressively up to 24 h (Fig. 1e, arrows) when morphological features typical of neurons were observed in most cells, a phenomenon that persisted longer than 48 h after treatment (not shown). Finally, as shown in Fig. 1f, after 5 d cell morphology resumed its original shape.

![Figure 1](https://academic.oup.com/ijnp/article-abstract/13/5/603/630229/607)
Immunocytochemistry

Using immunocytochemistry, we analysed the effect of imipramine exposure on the expression of different neural proteins including nestin, neurofilament, GFAP, NSE and synaptophysin. Figure 2(a–d) shows double staining for GFAP (red) and NF (green). Untreated astrocytes were positive only for the astrocytic marker GFAP (Fig. 2a), but co-expressing NF and GFAP cells could be observed after 8 h of imipramine treatment (Fig. 2b, arrow). Successively, some NF+/GFAP− cells were present at 24 h and persisted after 48 h of treatment (Fig. 2c, d, arrows), but then NF expression decreased and was completely absent after 5 d (not shown).

As shown in Fig. 2f, NSE expression was induced by 24 h imipramine treatment. Moreover, synaptophysin, a presynaptic vesicle protein absent in untreated astrocytes, was significantly expressed after imipramine treatment (Fig. 2c).

Similar effects were obtained with other antidepressants, i.e. fluoxetine and venlafaxine. In fact
both of these antidepressants were able to induce NF expression on astrocytes after 24 h of treatment (Fig. 2g, h).

In order to observe modification from the astrocytic to the neuronal phenotype, the co-localization of GFAP, NF and nestin was investigated by triple immunostaining (Fig. 3). Most untreated cells expressed both nestin and GFAP. Immunostaining for the neuronal marker NF was completely absent in untreated astrocytes, but was already induced in some cells after 8 h of treatment. Its expression progressively increased up to 24 h and persisted after 48 h of treatment. Data are expressed as percentage of cells immunopositive for GFAP, NF, nestin or their co-expression (mean ± S.E., n = 3).
treatment when about 15–20% of cells were positive, but then a decrease in staining intensity was registered, and after 5 d NF was almost completely absent. Nestin expression was not significantly modified by imipramine treatment: it was present in many untreated cells and persisted, roughly unchanged, throughout 5 d, although a slight decrease was observed between 8 and 24 h after treatment. The majority of differentiated cells showed co-localization of NF and GFAP and/or nestin (arrows), suggesting a more mature neuronal phenotype.
Cell proliferation

In order to identify the cell type(s) involved in proliferation, cells were assayed for BrdU incorporation and double-stained for NF. Results on BrdU incorporation are shown in Fig. 4. NF\(^+\) cells never showed labelled nuclei, suggesting that the appearance of NF-expressing cells cannot be ascribed to the proliferation of a small population of GFAP\(^+\) cells.

RT–PCR

To analyse the effect of imipramine treatment on neural gene expression we examined, using RT–PCR, the mRNA expression of some of the neural markers tested with immunocytochemistry (Fig. 5). Nestin mRNA was present in untreated astrocytes, but its levels significantly decreased after 2 h of treatment and then subsequently returned to control levels. Before treatment, astrocytes expressed high levels of GFAP mRNA, which remained unaltered after imipramine exposure. On the contrary, lower levels of NF-M and NSE mRNA were observed in untreated astrocytes. Cells showed a significant increase in NF-M mRNA after 8 h of imipramine treatment, whereas NSE mRNA required longer treatment in order to significantly increase.

Despite the high variability of RT–PCR results, we found statistically significant differences. This is due to the fact that all experiments showed the same trend, but with quantitative differences.

**Hes1 expression**

RT–PCR for Hes1 was performed to test the involvement of this bHLH (basic helix–loop–helix) gene in imipramine-induced differentiation. Hes1 is highly expressed by neural stem cells and is essential for maintaining neural precursor cells in an undifferentiated state. Hes1 was expressed at low levels in undifferentiated astrocytes, but its mRNA levels significantly increased after 2 h of treatment. mRNA expression returned to control levels as neuronal differentiation proceeded. In fact, after 24 and 48 h of treatment, it was not significantly different from that of untreated cells.

siRNA targeting Hes1 was used to treat astrocytes to specifically down-regulate Hes1 expression. Hes1 mRNA expression was measured by RT–PCR 14 d after transfection (Fig. 6\(a\)). As a result, cells showed decreased level of Hes1 mRNA expression for about 70%. In these cells, imipramine treatment was not able to induce an increase of NF expression (Fig. 6\(b\)). These results further confirm that Hes1 has a role in imipramine-induced differentiation of astrocytes into neuronal cells.

**Western blot**

Immunoblotting substantially confirmed immunocytochemistry results. In fact, as shown in Fig. 7, NF were absent in untreated cells, but their expression increased between 2 and 48 h after imipramine treatment, and then returned to control levels. Before treatment, astrocytes expressed both GFAP and nestin,
whose levels were not substantially modified by treatment.

**Whole-cell electrophysiological recording**

We performed whole-cell current-clamp experiments in imipramine-treated and -untreated astrocytes and, for comparison, in rat hippocampal neurons, in order to assess changes in the electrophysiological properties associated with phenotypical modification. We did not observe any notable change in the electrophysiological membrane properties with respect to control cells (see Supplementary Fig. S1, available online).

**Discussion**

The results of this study demonstrate that the antidepressant imipramine can induce astrocytes to differentiate in vitro into cells with a neuronal phenotype. After treatment with imipramine, cultured astrocytes acquired neuronal morphology and expressed neuronal markers. Similar results were obtained with fluoxetine and venlafaxine. These data suggest that differentiation of astrocytes into neurons might supply a crucial contribution to antidepressant-induced neurogenesis.

Our findings are consistent with those of recent studies reporting that adult neural stem cells exhibit structural and biological markers of astrocytes (Laywell *et al.* 2000; Skogh *et al.* 2001; Steindler *et al.* 2003). Moreover, we recently demonstrated that human cultured astrocytes can be induced, using a specific treatment, to differentiate into cells with a neuronal phenotype (Pillai *et al.* 2006). Since neurogenesis occurs only in specific brain regions it is not clear whether only a specific population of astrocytes can act as stem cells or if the local microenvironment of the neurogenic zone (SVZ and SGZ) strongly influences the behaviour of astrocytes, inducing them to differentiate into neurons. The former hypothesis could explain the relatively low percentage (about 15–20%) of differentiating cells, suggesting in our cultures the presence of type B astrocyte cells reported in adult proliferating brain areas (Kriegstein & Alvarez-Buylla, 2009).

Several authors have recently focused their attention on understanding the putative role of glial cells in the physiopathology of depressive disorders as well as in the mechanisms of action of antidepressant drugs (Cotter *et al.* 2001; Coyle & Schwarz, 2000; Ongür & Heckers, 2004; Páv *et al.* 2008). These researches were mainly focused on the involvement of astrocytes in the regulation of synaptic plasticity (Fuchs *et al.* 2004), induction of neurogenesis by neurotrophic factor release (Russo-Neustadt & Chen, 2005) and modulation of neurotransmitter uptake from synaptic cleft. However, to our knowledge, the present study is the first to show an effect of antidepressants on the differentiation of astrocytes into neuronal cells.

In addition to imipramine, we treated astrocytes with two different antidepressants: fluoxetine, a selective serotonin reuptake inhibitor (SSRI) and venlafaxine, a serotonin-norepinephrine reuptake inhibitor (SNRI). Both these novel antidepressants, as well as imipramine, work by blocking the reuptake of neurotransmitters such as norepinephrine and serotonin. All antidepressants had the same effect on astrocytes.

In our model, imipramine-induced differentiation was transient: 5 d after treatment, cell morphology reverted to its original astrocyte shape, and the expression of neuronal markers dramatically decreased. These observations might suggest that our experimental conditions are not sufficient to obtain stable differentiation to mature neurons. We may argue that the hippocampal microenvironment plays a pivotal role in this process, supporting the epigenetic factors necessary to complete the differentiation process.

Since in our model astrocytes were obtained from fetal brains, it may be objected that neuronal precursors or undifferentiated cells could be present in our cultures, themselves giving rise to cells with a neuronal phenotype. However, we observed the typical phenotype of differentiated astrocytes, expressing both GFAP and S100, in more than 90% of cells. In fact, cells were grown for at least 30–40 d with fetal calf serum before treatment, inducing differentiation of stem cells or neuronal precursor, as previously observed (Bottai *et al.* 2003). Since 10% of cells are GFAP negative, it could be hypothesized that they represent a population of undifferentiated cells that may be induced by treatment to differentiate into cells with a neuronal phenotype. However, we observed that the percentage of GFAP-negative cells did not decrease as a result of the appearance of NF-labelled cells, suggesting either proliferation of these cells or their lack of involvement in differentiation. To resolve any doubts, we performed BrdU incorporation during antidepressant treatment and double staining with BrdU/NF. Results showed no BrdU/NF co-localization, suggesting that neurofilament-positive cells derived from astrocytes.

We observed an early transient decrease in nestin mRNA, but no significant variation in the protein
expression during treatment. The presence of this protein in untreated cells does not contrast with what we stated above. In fact, although nestin is currently used as a marker of neural stem cells and its expression is expected to decrease with cell differentiation and mature phenotype acquisition, Sergent-Tanguy et al. (2006) observed a high percentage (about 80%) of cells co-expressing GFAP and nestin over 8 wk in primary cultures of rat astroglial cells. A long-lasting expression of this protein could be explained by the fact that astrocytes in culture exhibit many features of reactive glial cells (Wu & Schwartz, 1998). In several experimental models of injury to the CNS, nestin expression has been observed in reactive astroglial cells in the adult rat (Duggal et al., 1997; Krum & Rosenstein, 1999; Nakamura et al. 2004).

Antidepressant treatment induced the expression of synaptophysin, a presynaptic vesicle protein, indicating that these cells may have the ability to make synaptic connections. Cytoplasmic distribution of this protein is not surprising, since similar localization was observed in immature neurons during development (Diaz & Diana, 1992).

Changes in morphology and in neuronal markers induced by imipramine treatment were not accompanied by parallel changes in the electrophysiological properties. In fact we did not observe any notable change in the electrophysiological membrane properties with respect to control cells. This may suggest that our experimental conditions are not sufficient to obtain functional neurons.

Imipramine treatment induced a rapid increase in Hes-1 mRNA, followed by its decrease. This observation is consistent with the function of this transcription factor, which during brain development inhibits neurogenesis and maintains neural progenitors in an undifferentiated state (Ishibashi et al. 1995) and it is supported by knock-down experiments. Our results suggest that Hes1 increase was associated with astrocyte de-differentiation, while the subsequent decrease in its expression was necessary to induce differentiation into cells with a neuronal phenotype, as observed in vivo during development (Ross et al. 2003).

It has been shown that the cAMP signalling pathway plays a pivotal role in the pathophysiology of depression and in the action of antidepressants. This signal transduction pathway includes cAMP-dependent protein kinase (PKA) and cAMP response element-binding (CREB), involved in neuronal survival and differentiation and in synaptic plasticity (Frechilla et al. 1998; Mayr & Montminy, 2001; Nibuya et al. 1996). Clinical studies on autopsy brain specimens have shown a decrease in CREB levels in the temporal cortex of untreated depressive patients, whereas it significantly increased in antidepressant-treated patients (Dowlatshahi et al. 1998). We recently demonstrated that astrocytes can be induced in vitro to differentiate into cells with a neuronal marker by means of a differentiation cocktail containing, among other factors, IBMX and forskolin, which activate the cAMP–PKA signalling pathway (Pillai et al. 2006).

In conclusion, our results provide evidence that human astrocytes may play an active role in antidepressant-induced neurogenesis, by acting as neural stem cells and differentiating into cells with a neuronal phenotype. Further studies are needed to examine the in-vivo effects of antidepressants on type B astrocytes. A better understanding of the molecular mechanisms of this process will lead to establishing the role of astrocytes as a target for the development of new antidepressants.

Note

Supplementary material accompanies this paper on the Journal’s website (http://journals.cambridge.org/pnp).

Acknowledgements

The authors thank Dr Mary Groeneweg for the English editing of the manuscript.

Statement of Interest

None.

References


Brezun JM, Daszuta AA (1999). Depletion in serotonin decreases neurogenesis in the dentate gyrus and the


