Modulation of neuroplastic molecules in selected brain regions after chronic administration of the novel antidepressant agomelatine

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Abstract

Rationale Neuronal plasticity is associated with depression, probably as a result of modified expression of proteins important for cellular resiliency. It is therefore important to establish if and how antidepressant drugs may be able to regulate these mechanisms in order to achieve relevant clinical effects.

Objective We investigated the effects of chronic treatment with agomelatine (an MT_1/MT_2 receptor agonist and $5-HT_{2C}$ receptor antagonist) on the brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF-2), and activity-regulated cytoskeleton-associated protein (Arc).

Methods Animals were treated for 21 days with agomelatine, venlafaxine, or a vehicle and sacrificed 1 h (6 p.m.) or 16 h after the last injection (9 a.m.) to evaluate the messenger RNA (mRNA) and protein expression of these neuroplastic markers in the hippocampus and prefrontal cortex.

Results Agomelatine, but not venlafaxine, produced major transcriptional changes in the hippocampus, where significant up-regulations of BDNF and FGF-2 were observed. Both drugs up-regulated the Arc transcription levels. No effects were observed in the prefrontal cortex. Instead, the levels of BDNF protein were elevated by agomelatine in both regions: the effects of the drug on mRNA levels in the hippocampus and cortex are different, while the effects on the protein seem to have the same cumulative result, suggesting different modulatory mechanisms in the two regions.

Conclusions Our data provide new information regarding the molecular mechanisms that contribute to the chronic effects of the new antidepressant agomelatine on brain function. The ability of agomelatine to modulate the expression of these neuroplastic molecules, which follows a circadian rhythm, may contribute to its antidepressant action.

Keywords BDNF · FGF-2 · Arc · Depression · Plasticity · Melatonergic system · Serotonin · Trophic factor · IEG

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Introduction

During the last decade, the clinical efficacy of antidepressant drugs has been associated with long-term neuroadaptative changes that may take place in selected brain structures (Berton and Nestler 2006; Castren 2005). Several studies have shown that the hippocampus is altered in depressed subjects and that these alterations are associated,

in many cases, with a poor clinical outcome (Macqueen and Frodl 2010). Indeed, different studies report a reduced volume of this structure in depressed patients (Frodl et al. 2002), and there seems to be a relation between this volume reduction and the time during which the depressive episodes went untreated (Sheline et al. 2003). These results support the hypothesis that the hippocampus and its connections within limbic–cortical networks may play a crucial role in the pathogenesis of major depression. One mechanism that has been extensively studied in recent years is the regulation of the neurotrophin brain-derived neurotrophic factor (BDNF), whose gene and protein expression and function may be defective in mood disorders (Calabrese et al. 2009; Groves 2007; Kozisek et al. 2008; Martinowich et al. 2007). The modulation of BDNF represents a key element in long-term adaptive changes brought about by antidepressant drugs and may contribute to normalize neuroplastic defects associated with depression.

On this basis, it is important to establish which mechanisms may prove effective in the regulation of this neurotrophin in specific brain regions. So far, studies have focused on antidepressant drugs that share similar mechanisms, acting specifically on molecules that increase the levels of monoamines (serotonin and noradrenaline) in the synaptic cleft. These studies suggest that not all antidepressants behave in the same way, since complex regulatory mechanisms take place following prolonged drug administration, depending on the timing and on the brain region examined. However, some evidence suggest that drugs that act on more than one neurotransmitter system, such as the serotonin–norepinephrine reuptake inhibitor (SNRI) duloxetine, may be more effective than those that exert a major effect only on the serotonergic system (Calabrese et al. 2007; Molteni et al. 2009). On the other hand, little information is currently available on the potential effect of antidepressant drugs that work without inhibiting monoamine transporters. We thus decided to investigate the action of venlafaxine (SNRI) and agomelatine, a novel antidepressant that has a unique receptor profile as a MT_1/MT_2 melatonergic agonist (Audinot et al. 2003) and $5-HT_{2C}$ receptor antagonist (Millan et al. 2003). These receptor components can determine independent as well as interacting activities, useful for normalizing abnormalities associated with mood disorders, since they are particularly relevant for stabilizing disturbances in circadian rhythms, which are associated with depressive illness (Pandi-Perumal et al. 2006).

Interestingly, the expression of BDNF is also influenced by light and dark cycles in rats (Bova et al. 1998; Liang et al. 1998) as well as in humans (Begliuomini et al. 2008). We have recently demonstrated that an acute injection of agomelatine can up-regulate the expression of BDNF messenger RNA (mRNA) levels in the prefrontal cortex

through the functional interaction between melatonergic MT_1/MT_2 and serotonergic $5-HT_{2C}$ receptors (Molteni et al. 2010), thus preventing the circadian down-regulation of the neurotrophin.

Based on this observation, in the present study, we investigated the effects produced by chronic agomelatine treatment, in comparison to the antidepressant drug venlafaxine, on the mRNA and protein expression of the neurotrophin BDNF. We have also considered the modulation of basic fibroblast growth factor (FGF-2), another trophic molecule whose gene expression may be reduced in the depressed brain (Riva et al. 2005; Turner et al. 2006) and that can be enhanced by antidepressant treatment (Bachis et al. 2008). Lastly, we studied the regulation of the activity-regulated gene (Arc), whose mRNA levels are also modulated by antidepressant treatment in specific brain regions (Molteni et al. 2008) and are closely related to BDNF (Bramham and Messaoudi 2005).

Methods

Materials

General reagents were purchased from Sigma–Aldrich (Milan, Italy), and molecular biology reagents were obtained from Ambion (Austin, TX, USA), Roche (Monza, Italy), Bio-Rad (Milan, Italy), Amersham Life Science (Milan, Italy), Santa Cruz Biotechnology (Tebu-bio, Milan, Italy), and Cell-Signaling (Euroclone, Pero, Milan, Italy).

Animals and drug treatment

Sprague–Dawley rats (225–250 g) were purchased from Charles River (Calco, Italy). Animals were kept at constant temperature (22°C) with a regular 12-h light/dark cycle (light-on at 7 a.m.). The rats were housed in groups of four with ad libitum access to food and water for 2 weeks before the treatment. The rats were treated chronically (21 days) with agomelatine (40 mg/kg i.p.), the reference antidepressant drug venlafaxine (10 mg/kg i.p.), or a vehicle (hydroxyethylcellulose, 1%, 1 ml/kg i.p.). The choice of agomelatine dose was made on the basis of their activity at this range in animal models of depression and anxiety (Papp et al. 2003) and on neurogenesis (Banasr et al. 2006). Agomelatine and reference antidepressant treatments were given at 5 p.m. (2 h before the start of the dark cycle).

Rats were killed by decapitation 1 h (6 p.m.) or 16 h later (9 a.m.) after the last injection, in order to evaluate both the persistency of the effects produced by chronic drug administration, as well as the potential impact on the circadian changes in the expression of neuroplastic genes (Molteni et al. 2010). The hippocampus was dissected from

the whole brain, whereas the prefrontal cortex (defined as Cg1, Cg3, and IL subregions corresponding to plates 6–10 according to the atlas by Paxinos and Watson) was dissected from 2-mm-thick slices (Paxinos and Watson 1996). The brain specimens were frozen on dry ice and stored at −80°C for further analysis. All animal handling and experimental procedures were performed in accordance with the EC guidelines (EEC Council Directive 86/609, 1987) and the Italian legislation on animal experimentation (Decreto Legislativo 116/92).

RNA preparation

Tissue from different brain structures was homogenized in 4 M guanidinium isothiocyanate (containing 25 mM sodium citrate, pH 7.5; 0.5% sarcosyl; and 0.1% 2-mercaptoethanol). Total RNA was isolated by phenol/chloroform extraction, and quantification was carried out by spectrophotometric analysis.

RNase protection assay

A transcription kit (MAXI script; Ambion) was used to generate cRNA probes, and ^{32}P-CTP was utilized as a radiolabeled nucleotide. The cRNA probes and the relative protected fragments (pf) were as follows: BDNF=800 bp, pf=740; FGF-2=524 bp, pf=477; Arc=630 bp, pf=620; b-actin (pTRI-b-actin-rat, Ambion)=164 bp, pf=126 bp. The cRNA probe of BDNF was complementary to exon IX, which is common to all neurotrophin transcripts (Aid et al. 2007).

The RNase protection assay was carried out on a 10-µg sample of total RNA, as described previously (Calabrese et al. 2007). Briefly, after ethanol precipitation, total RNA was dissolved in 20 µl of hybridization solution (80% formamide; 40 mM PIPES, pH 6.4; 400 mM sodium acetate, pH 6.4; and 1 mM EDTA) containing 150,000 cpm of ^{32}P-labeled BDNF, FGF-2, Arc cRNA probes (specific activity >10^8 cpm/µg), and 50,000 cpm of ^{32}P-labeled β-actin probe. After being heated at 85°C for 10 min, the cRNA probes were allowed to hybridize to the endogenous RNAs at 45°C overnight.

After the hybridization, the solution was diluted with 200 µl of RNase digestion buffer (300 mM NaCl; 10 mM Tris–HCl, pH 7.4; and 5 mM EDTA, pH 7.4) containing a 1:400 dilution of a RNase cocktail (1 mg/ml RNase A and 20 U/ml RNase T1) and incubated for 30 min at 30°C. Proteinase K (10 µg) and sodium dodecyl sulfate (SDS; 10 µl of 20% stock solution) were then added to the sample, and the mixture was incubated at 37°C for an additional 15 min. At the end of the incubation, the sample was extracted with phenol/chloroform and ethanol precipitated. The pellet containing the RNA–RNA hybrids was dried and resuspended in loading buffer (80% formamide, 0.1% xylene cyanol, 0.1% bromophenol blue, and 2 mM

EDTA), boiled at 95°C for 5 min, and separated on 5% polyacrylamide gel under denaturing conditions (7 M urea). The protected fragments were visualized by autoradiography.

Preparation of protein extract and Western blot analysis

Tissues were manually homogenized using a Dounce homogenizer in 600 µl of a pH-7.4 cold buffer (solution A) containing 0.32 M sucrose, 10 mM Tris–HCl, pH 7.4, and commercial cocktails of protease (cod. 11697498001, Roche) and phosphatase (cod. P5726, Sigma–Aldrich) inhibitors. The homogenate was centrifuged at 2,000×g for 1 min at 4°C, obtaining a pellet (P1) corresponding to the nuclear fraction, which was resuspended in the solution A buffer and centrifuged again (2,000×g for 2 min at 4°C). The supernatants, derived from the two centrifugation steps, were collected and centrifuged at 23,000×g for 5 min at 4°C to obtain a supernatant (S2, Cytosol) and a pellet (P2) corresponding to the crude synaptosomal fraction which was resuspended in the solution A buffer. Discontinuous Ficoll 400 (Sigma–Aldrich) gradients were prepared by layering 2 ml each in order 13% Ficoll 400 and 5% in solution A into 5-ml polycarbonate centrifuge tubes. The resuspended P2 pellet was then layered on top of a gradient and centrifuged at 45,000×g for 45 min at 4°C. After centrifugation, synaptosomes were collected (interphase between 13% and 5% Ficoll), diluted 1:10 with solution A buffer, and centrifuged at 23,000×g for 20 min at 4°C. The supernatant was removed, and the pellet (synaptosomes) was resuspended in 70 µl of solution A buffer. Total protein content was measured according to the Bradford Protein Assay procedure (Bio-Rad), using bovine serum albumin as the calibration standard.

In the present study, Western blot analysis was performed to investigate the levels of the precursor and mature BDNF proteins. After adjusting the total protein concentrations to the same amount (10 µg), all samples were run under reducing conditions on a 14% SDS polyacrilamide gel and then electrophoretically transferred onto PVDF membranes (Amersham Life Science). Blots were blocked with 10% nonfat, dry milk and then incubated with the primary antibody anti-BDNF polyclonal antibody able to recognize both the mature form of the neurotrophin (mBDNF, 14 kDa) and its precursor (proBDNF, 32 kDa; 1:1,000 for mBDNF and 1:500 for proBDNF, 4°C, overnight; Santa Cruz Biotechnology). Membranes were then incubated for 1 h at room temperature with a peroxidase-conjugated anti-rabbit IgG (Cell Signaling; 1:2,000), and immunocomplexes were visualized by chemiluminescence, utilizing the ECL Western Blotting Kit (Amersham Life science), according to the manufacturer's instructions. Results were standardized to the control protein β-actin, which was detected by evaluating the band

density at 43 kDa after probing the membranes with a polyclonal antibody (1:10,000 dilution; Sigma), followed by a 1:10,000 dilution of peroxidase-conjugated anti-mouse IgG (Sigma).

Densiometric and statistical analyses

The levels of mRNA and protein were calculated by measuring the optical density of the autoradiographic bands using Quantity One software (Bio-Rad). To ensure that autoradiographic bands were in the linear range of intensity, different exposure times were used. β-Actin was employed as the internal standard for the RNase protection assay and Western blotting because its expression was not regulated by chronic antidepressant treatment. Statistical comparisons were carried out by one-way analysis of variance (ANOVA). Significance for all tests was assumed at $p < 0.05$.

Data are presented as means±standard error (SEM), with each individual group composed of 6–8 samples. For graphic clarity, optical densities from experimental groups were expressed and presented as a mean percentage of the control group, namely, the group that received only the vehicle.

Results

We started by investigating the modulation of BDNF mRNA levels in the hippocampus and prefrontal cortex, two key brain regions for mood disorders. In the hippocampus, chronic agomelatine treatment increased the mRNA levels of BDNF 1 h (+27%, $p < 0.05$) and 16 h (+26%, $p < 0.05$) after the last administration, whereas venlafaxine did not produce any significant change on the neurotrophins' gene expression (Fig. 1a).

In the prefrontal cortex (Fig. 1b), in line with our recent results (Molteni et al. 2010), we found a "circadian" variation of BDNF mRNA levels, which were significantly lower in animals sacrificed 2 h after the light-on (inactive period, 9 a.m.) than 1 h before the light-off (active period, 6 p.m.; −34%, $p < 0.05$). In this area, chronic venlafaxine reduced the neurotrophins' mRNA levels at 1 h (−34%, $p < 0.05$), whereas agomelatine did not produce any significant change (−23%, $p > 0.05$). Conversely, both drugs did not alter BDNF gene expression at 16 h.

We next focused on analyzing the BDNF protein by Western Blot analysis in order to measure the levels of the neurotrophin precursor (proBDNF) and its mature form (mBDNF) in the cytosolic fraction (S2) and in the synaptosomal fraction (Syn). In the hippocampus (Fig. 2), in agreement with the transcriptional changes, chronic agomelatine treatment, but not venlafaxine, increased mBDNF levels in the cytosolic fraction 1 h (+66%, $p < 0.05$; Fig. 2a), but not 16 h (−12%, $p > 0.05$; Fig. 2a), after the last injection,
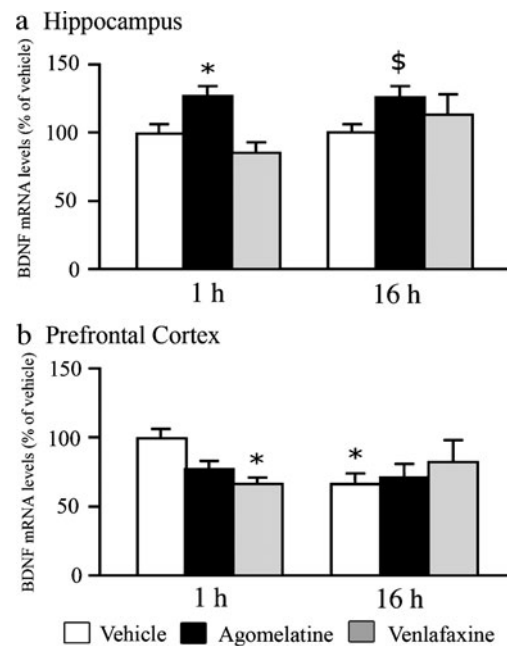

Fig. 1 Effect of chronic treatment with agomelatine or venlafaxine on BDNF mRNA levels in the hippocampus (**a**) and prefrontal cortex (**b**) of adult male rats. Animals were killed 1 h (6 p.m.) or 16 h (9 a.m.) after the last administration (5 p.m.). The data represent the mean of six independent determinations and are expressed as percentage of controls (animals treated with the vehicle, set at 100%). *$p < 0.05$ vs. vehicle, 1 h; $^{\$}p < 0.05$ vs. vehicle, 16 h (one-way ANOVA with Fisher's protected least significant difference (PLSD))

without affecting the levels of proBDNF (Fig. 2a). Conversely, no significant changes were observed in the synaptosomal fraction after agomelatine as well as venlafaxine administration, although a trend toward an increase was found with both drugs at 1 h (+24%, +22%, $p > 0.05$; Fig. 2b).

In the prefrontal cortex (Fig. 3), differently from transcriptional changes, mBDNF levels were increased in the cytosol after both agomelatine and venlafaxine at 1 h (+35%, $p < 0.05$; +41%, $p < 0.05$; Fig. 3a). Interestingly, mBDNF protein levels show an opposite circadian profile to mRNA levels, since they were higher 2 h after the light-on than 1 h before the light-off in the cytosolic fraction, although the effect does not reach statistical significance (+35%, $p = 0.118$; Fig. 3a). Moreover, agomelatine up-regulated the levels of the neurotrophin precursor 16 h after the end of the treatment in the cytosolic fraction (+50%, $p < 0.05$; Fig. 3a). In the synaptosomal compartment, agomelatine increased proBDNF levels at both the time points (1 h, +44%, $p < 0.05$; 16 h, +82%, $p < 0.01$; Fig. 3b), whereas venlafaxine increased proBDNF only at 16 h (1 h, −35%, $p > 0.05$; 16 h, +77%, $p < 0.01$; Fig. 3b).

With regard to FGF-2, we found that chronic agomelatine treatment increased hippocampal mRNA levels of the trophic factor (Fig. 4a) 1 h (+33%, $p < 0.01$) as well as 16 h (+22%, $p < 0.05$) after the last administration, whereas venlafaxine did not produce any significant change. In the

Fig. 2 Effect of chronic treatment with agomelatine or venlafaxine on BDNF protein levels in the hippocampus of adult male rats. Western blot analysis was performed on the cytosolic (**a**, 1 h, 16 h) of the synaptosomal fraction (**b**, 1 h, 16 h). The data represent the mean of four independent determinations and are expressed as percentage of controls (animals treated with the vehicle, set at 100%). *$p<0.05$ vs. vehicle, 1 h (one-way ANOVA with Fisher's, PLSD)
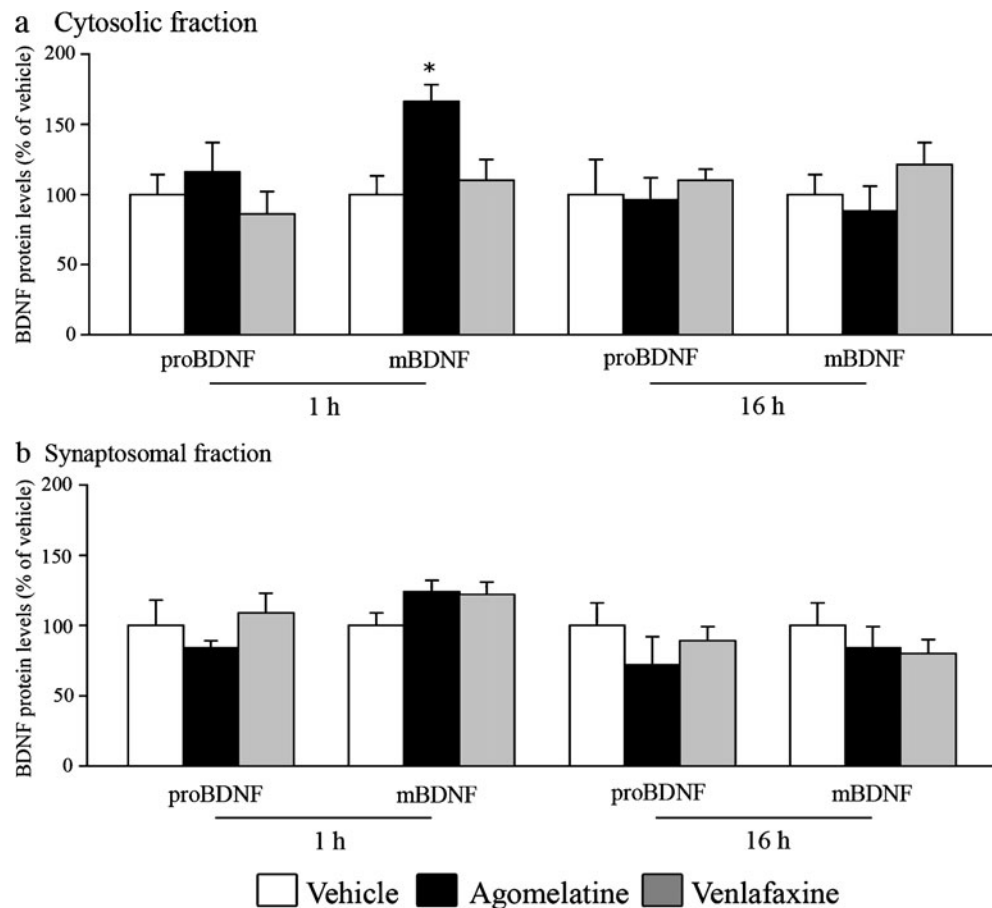
prefrontal cortex (Fig. 4b), similar to BDNF mRNA levels, FGF-2 gene expression was significantly lower 2 h after the light-on than 1 h before the light-off (-33%, $p<0.01$). Chronic venlafaxine reduced the expression of FGF-2 mRNA levels (-23%, $p<0.05$) at 1 h postinjection, whereas agomelatine did not produce any significant change.

Finally, we investigated the gene expression of the activity-regulated gene *Arc*, whose changes may parallel the modifications of BDNF (Molteni et al. 2008). We found that in the hippocampus, Arc mRNA levels were significantly up-regulated 1 h after the last injection of agomelatine ($+63\%$, $p<0.01$) and venlafaxine ($+42\%$, $p<0.05$; Fig. 5a). In the prefrontal cortex, we found a significant difference of Arc mRNA levels in the animals sacrificed 2 h after the light-on than 1 h before the light-off (-32%, $p<0.05$; Fig. 5b). In this area, Arc mRNA levels appeared down-regulated at 1 h by chronic administration of both drugs, an effect significant only with venlafaxine (-30%, $p<0.05$; Fig. 5b), whereas no changes were found at 16 h.

Discussion

Our results demonstrate that chronic treatment with agomelatine, but not venlafaxine, increases the expressions

of BDNF and FGF-2, two neurotrophic factors that are implicated in mood disorders and whose modulation may contribute to the action of antidepressants (Bachis et al. 2008; Calabrese et al. 2009). These data provide further support to the notion that prolonged treatment with these drugs produces adaptive changes at the hippocampal and cortical levels, increasing proteins that are important for neuronal plasticity.

With regard to BDNF transcription, major changes were found in the hippocampus, where, after chronic treatment, agomelatine produces a stable increase of the neurotrophin mRNA levels 1 and 16 h after the last drug administration. Conversely, venlafaxine did not produce any significant change at both time points, probably because at the dose used in the present study, venlafaxine acts primarily as a SSRI (Bymaster et al. 2001). This might explain its relative inefficacy in regulating BDNF expression that appears to be modulated more efficiently by drugs acting on multiple neurotransmitter systems (Calabrese et al. 2007). Moreover, although there are reports of enhanced expression of BDNF after venlafaxine, these results may often differ, based on experimental paradigms, doses, time of sacrifice, and methods used (Cooke et al. 2009; Kozisek et al. 2008; Larsen et al. 2010). To this regard, one major point that has to be considered regarding our work is that all animals were

Fig. 3 Effect of chronic treatment with agomelatine or venlafaxine on BDNF protein levels in the prefrontal cortex of adult male rats. Western blot analysis was performed on the cytosolic (**a**, 1 h, 16 h) of the synaptosomal fraction (**b**, 1 h, 16 h). The data represent the mean of four independent determinations and are expressed as percentage of controls (animals treated with the vehicle, set at 100%). *$p < 0.05$ vs. vehicle, 1 h; $^{\$}p < 0.05$; and $^{\$\$}p < 0.01$ vs. vehicle, 16 h (one-way ANOVA with Fisher's PLSD)
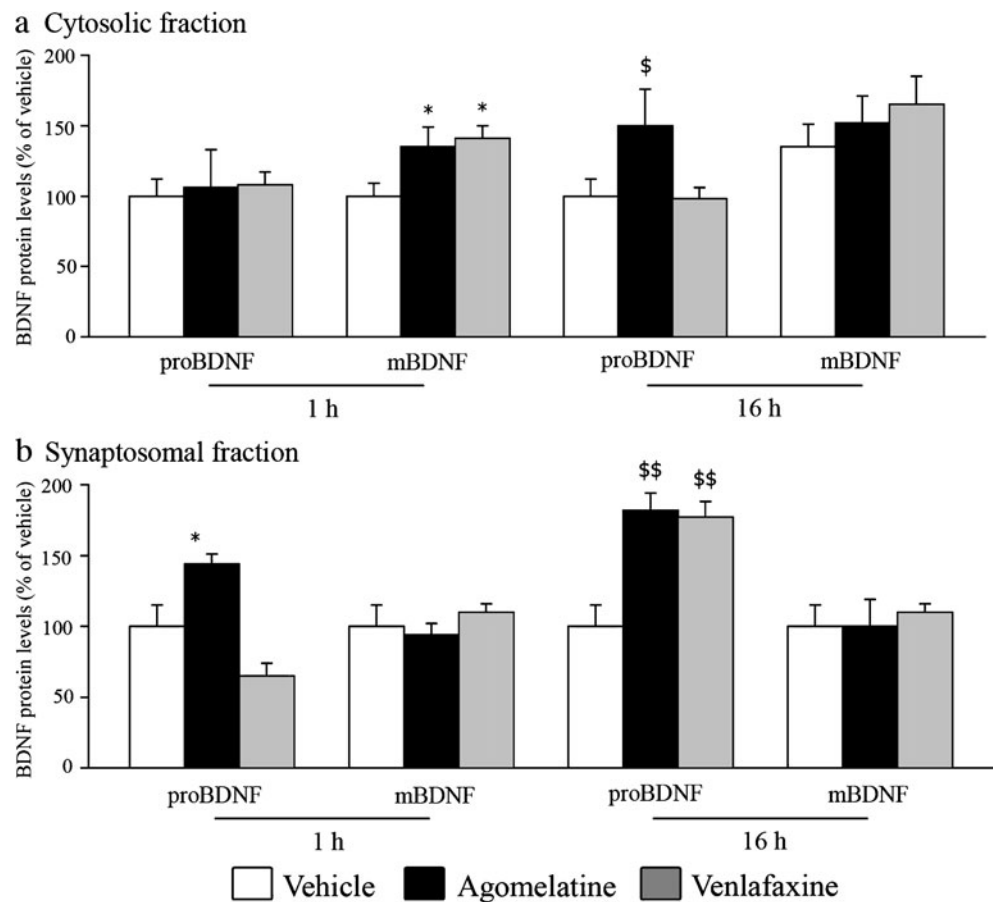
treated in the late afternoon, a time considered most appropriate for agomelatine activity according to its melatonergic properties. Even if the exact mechanism underlying the agomelatine effects on BDNF has not been analyzed in the present study, data from Soumier et al. (2009; that show BDNF protein increase in the hippocampus under chronic conditions), as well as those recently published by our team (showing increased BDNF mRNA levels under acute conditions in the prefrontal cortex; Molteni et al. 2010), suggest that in our experimental conditions, agomelatine may modulate BDNF through the combination of the activation of MT_1/MT_2 melatonergic receptors and the blockade of $5-HT_{2C}$ receptors. Nevertheless, even if Soumier and collaborators (Soumier et al. 2009) did not show BDNF protein increase after treatment with two different $5-HT_{2C}$ antagonists, it has been recently demonstrated that chronic treatment with the selective $5-HT_{2C}$ antagonist S32006 can increase BDNF expression in the hippocampal dentate gyrus (Dekeyne et al. 2008). Conversely, a melatonin receptor agonist does not alter BDNF mRNA levels in cultured cerebellar granule cells, but increases BDNF protein (Imbesi et al. 2008). It is also plausible that the combination of both mechanisms (i.e., blockade of $5-HT_{2C}$ and activation of MT_1/MT_2) is needed to exert an effect on BDNF mRNA levels.

In line with the mRNA changes, the levels of mature BDNF (mBDNF) are significantly increased in the hippocampus of agomelatine-treated rats, whereas no effect was observed with venlafaxine. However, such increase is observed in the cytosolic compartment 1 h after the last administration of agomelatine but not 16 h later or in the synaptosomal fraction. Hence, it may be hypothesized that agomelatine determines a stable increase of the mRNA levels of the neurotrophin and that its administration under chronic conditions may produce a transient increase of BDNF translation. We should also bear in mind that other parameters, including time of injection and sacrifice, as well as the methodology for protein analysis, can account for differences between the results of the present study and those of previous reports (Calabrese et al. 2007; Soumier et al. 2009).

The regulation of BDNF in the prefrontal cortex is somewhat different. In fact, 1 h after the injection, mRNA levels were not affected by chronic treatment with agomelatine, but were reduced by venlafaxine. No significant change was found with both drugs at 16 h with respect to their time-matched control, although BDNF expression was significantly lower in saline-injected animals at 16 h when compared to the rats killed at 1 h. These data confirm our recent observation under acute conditions (Molteni et
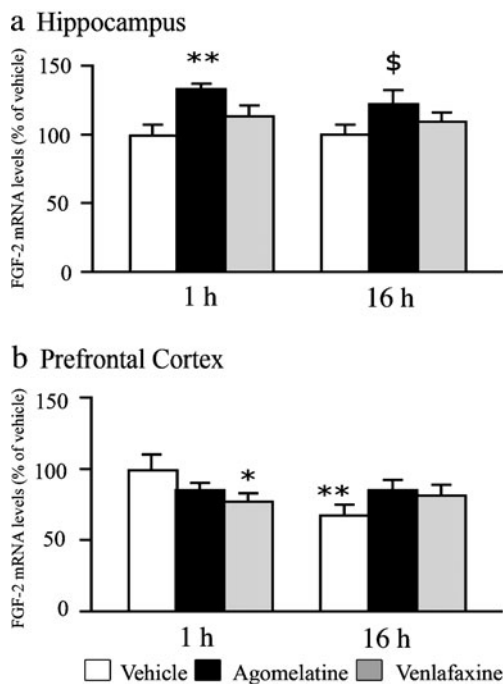
Fig. 4 Effect of chronic treatment with agomelatine or venlafaxine on FGF-2 gene expression in the hippocampus (**a**) and prefrontal cortex (**b**) of adult male rats. Animals were killed 1 h (6 p.m.) or 16 h (9 a.m.) after the last administration (5 p.m.). The data represent the mean of five independent determinations and are expressed as percentage of controls (animals treated with vehicle, set at 100%). *$p<0.05$ and **$p<0.01$ vs. vehicle, 1 h; $^{\$}p<0.05$ vs. vehicle, 16 h (one-way ANOVA with Fisher's PLSD)

al. 2009) and suggest that, within the prefrontal cortex, there is a circadian variation in the transcriptional profile of the neurotrophin. Despite the lack of changes in BDNF mRNA levels, we found that agomelatine as well as venlafaxine were able to increase BDNF protein, suggesting that in this brain region, the two drugs may affect BDNF at a posttranscriptional level. The dissociation between mRNA and protein levels is also suggested by the observation that, despite a circadian variation of BDNF mRNA levels, which are higher in the 1 h before the light-off, we did not find similar changes in BDNF protein, which actually appears to be somewhat higher 2 h after the light-on. On the other hand, a discrepancy between mRNA and protein levels is not surprising (Pollock et al. 2001). In fact, the amount of mRNA is determined by its rate of synthesis versus its rate of degradation (Jacobshagen et al. 2008), whereas the increase in protein could be due to an increase of packaging within vesicles, retention of locally synthesized BDNF secretion, or altered BDNF transport. However, since multiple BDNF transcripts have been described (Aid et al. 2007), we cannot rule out the possibility that the protein changes in different subcellular compartments could be sustained by the modulation of specific transcripts.

Hippocampal FGF-2 expression was similarly up-regulated by agomelatine and not by venlafaxine. We have previously demonstrated that FGF-2 mRNA levels are increased in rat hippocampus, following repeated administration of fluoxetine in combination with olanzapine, but not with the two drugs alone (Maragnoli et al. 2004). This suggests again the possibility that a combination of different receptor mechanisms may be more effective, under chronic conditions, in the modulation of neurotrophic factors which participate in neuroplastic events that take place in specific brain regions.

The modulation of the activity-regulated gene *Arc* only partly overlaps the results obtained with BDNF. Within the hippocampus, there is a transient up-regulation of its gene expression 1 h after the last injection of agomelatine as well as venlafaxine, in line with data obtained using other antidepressants (Molteni et al. 2008). Consistent with the circadian variation of BDNF mRNA levels in the prefrontal cortex, Arc and FGF-2 gene expression undergoes similar changes.

In summary, our data provide new information regarding the molecular mechanisms that contribute to the chronic effects of the new antidepressant agomelatine, highlighting its ability to modulate the expression of neuroplastic molecules, probably through its action on melatonergic and 5-HT$_{2C}$ receptors. Although additional studies are
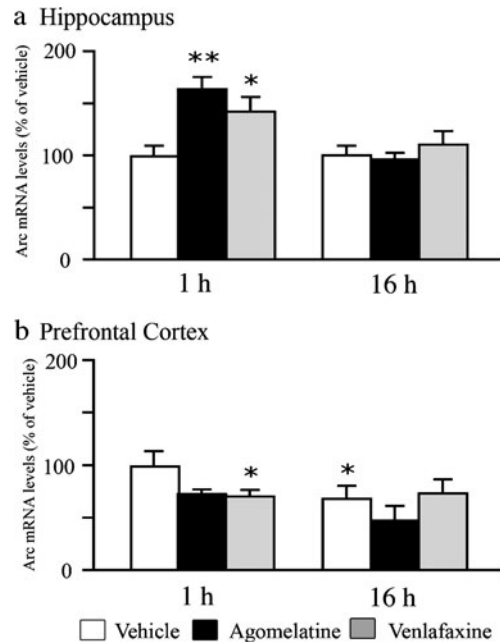

Fig. 5 Effect of chronic treatment with agomelatine or venlafaxine on *Arc* gene expression in the hippocampus (**a**) and prefrontal cortex (**b**) of adult male rats. Animals were killed 1 h (6 p.m.) or 16 h (9 a.m.) after the last administration (5 p.m.). The data represent the mean of five independent determinations and are expressed as percentage of controls (animals treated with the vehicle, set at 100%). *$p<0.05$ and **$p<0.01$ vs. vehicle, 1 h (one-way ANOVA with Fisher's PLSD)

required to further investigate the significance of the anatomical and temporal differences of agomelatine effects, its ability to up-regulate neuroplastic molecules, whose expression follows a circadian rhythm, may contribute to the chronic effects produced by agomelatine on brain function.

References

Aid T, Kazantseva A, Piirsoo M, Palm K, Timmusk T (2007) Mouse and rat BDNF gene structure and expression revisited. J Neurosci Res 85:525–535

Audinot V, Mailliet F, Lahaye-Brasseur C, Bonnaud A, Le Gall A, Amosse C, Dromaint S, Rodriguez M, Nagel N, Galizzi JP, Malpaux B, Guillaumet G, Lesieur D, Lefoulon F, Renard P, Delagrange P, Boutin JA (2003) New selective ligands of human cloned melatonin MT1 and MT2 receptors. N Schmied Arch Pharmacol 367:553–561

Bachis A, Mallei A, Cruz MI, Wellstein A, Mocchetti I (2008) Chronic antidepressant treatments increase basic fibroblast growth factor and fibroblast growth factor-binding protein in neurons. Neuropharmacology 55:1114–1120

Banasr M, Soumier A, Hery M, Mocaer E, Daszuta A (2006) Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. Biol Psychiatry 59:1087–1096

Begliuomini S, Lenzi E, Ninni F, Casarosa E, Merlini S, Pluchino N, Valentino V, Luisi S, Luisi M, Genazzani AR (2008) Plasma brain-derived neurotrophic factor daily variations in men: correlation with cortisol circadian rhythm. J Endocrinol 197:429–435

Berton O, Nestler EJ (2006) New approaches to antidepressant drug discovery: beyond monoamines. Nat Rev Neurosci 7:137–151

Bova R, Micheli MR, Qualadrucci P, Zucconi GG (1998) BDNF and trkB mRNAs oscillate in rat brain during the light-dark cycle. Brain Res Mol Brain Res 57:321–324

Bramham CR, Messaoudi E (2005) BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis. Prog Neurobiol 76:99–125

Bymaster FP, Dreshfield-Ahmad LJ, Threlkeld PG, Shaw JL, Thompson L, Nelson DL, Hemrick-Luecke SK, Wong DT (2001) Comparative affinity of duloxetine and venlafaxine for serotonin and norepinephrine transporters in vitro and in vivo, human serotonin receptor subtypes, and other neuronal receptors. Neuropsychopharmacology 25:871–880

Calabrese F, Molteni R, Maj PF, Cattaneo A, Gennarelli M, Racagni G, Riva MA (2007) Chronic duloxetine treatment induces specific changes in the expression of BDNF transcripts and in the subcellular localization of the neurotrophin protein. Neuropsychopharmacology 32:2351–2359

Calabrese F, Molteni R, Racagni G, Riva MA (2009) Neuronal plasticity: a link between stress and mood disorders. Psychoneuroendocrinology

Castren E (2005) Is mood chemistry? Nat Rev Neurosci 6:241–246

Cooke JD, Grover LM, Spangler PR (2009) Venlafaxine treatment stimulates expression of brain-derived neurotrophic factor protein in frontal cortex and inhibits long-term potentiation in hippocampus. Neuroscience 162:1411–1419

Dekeyne A, Mannoury la Cour C, Gobert A, Brocco M, Lejeune F, Serres F, Sharp T, Daszuta A, Soumier A, Papp M, Rivet JM, Flik G, Cremers TI, Muller O, Lavielle G, Millan MJ (2008) S32006, a novel 5-HT2C receptor antagonist displaying broad-based antidepressant and anxiolytic properties in rodent models. Psychopharmacol Berl 199:549–568

Frodl T, Meisenzahl EM, Zetzsche T, Born C, Groll C, Jager M, Leinsinger G, Bottlender R, Hahn K, Moller HJ (2002) Hippocampal changes in patients with a first episode of major depression. Am J Psychiatry 159:1112–1118

Groves JO (2007) Is it time to reassess the BDNF hypothesis of depression? Mol Psychiatry 12:1079–1088

Imbesi M, Uz T, Dzitoyeva S, Manev H (2008) Stimulatory effects of a melatonin receptor agonist, ramelteon, on BDNF in mouse cerebellar granule cells. Neurosci Lett 439:34–36

Jacobshagen S, Kessler B, Rinehart CA (2008) At least four distinct circadian regulatory mechanisms are required for all phases of rhythms in mRNA amount. J Biol Rhythms 23:511–524

Kozisek ME, Middlemas D, Bylund DB (2008) Brain-derived neurotrophic factor and its receptor tropomyosin-related kinase B in the mechanism of action of antidepressant therapies. Pharmacol Ther 117:30–51

Larsen MH, Mikkelsen JD, Hay-Schmidt A, Sandi C (2010) Regulation of brain-derived neurotrophic factor (BDNF) in the chronic unpredictable stress rat model and the effects of chronic antidepressant treatment. J Psychiatr Res 44:808–816

Liang FQ, Walline R, Earnest DJ (1998) Circadian rhythm of brain-derived neurotrophic factor in the rat suprachiasmatic nucleus. Neurosci Lett 242:89–92

Macqueen G, Frodl T (2010) The hippocampus in major depression: evidence for the convergence of the bench and bedside in psychiatric research? Mol Psychiatry

Maragnoli ME, Fumagalli F, Gennarelli M, Racagni G, Riva MA (2004) Fluoxetine and olanzapine have synergistic effects in the modulation of fibroblast growth factor 2 expression within the rat brain. Biol Psychiatry 55:1095–1102

Martinowich K, Manji H, Lu B (2007) New insights into BDNF function in depression and anxiety. Nat Neurosci 10:1089–1093

Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V, Rivet JM, Cussac D (2003) The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine2C receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. J Pharmacol Exp Ther 306:954–964

Molteni R, Calabrese F, Mancini M, Racagni G, Riva MA (2008) Basal and stress-induced modulation of activity-regulated cytoskeletal associated protein (Arc) in the rat brain following duloxetine treatment. Psychopharmacol Berl 201:285–292

Molteni R, Calabrese F, Cattaneo A, Mancini M, Gennarelli M, Racagni G, Riva MA (2009) Acute stress responsiveness of the neurotrophin BDNF in the rat hippocampus is modulated by chronic treatment with the antidepressant duloxetine. Neuropsychopharmacology 34:1523–1532

Molteni R, Calabrese F, Pisoni S, Gabriel C, Mocaer E, Racagni G, Riva MA (2010) Synergistic mechanisms in the modulation of the neurotrophin BDNF in the rat prefrontal cortex following acute agomelatine administration. World J Biol Psychiatry 11:148–153

Pandi-Perumal SR, Srinivasan V, Cardinali DP, Monti MJ (2006) Could agomelatine be the ideal antidepressant? Expert Rev Neurother 6:1595–1608

Papp M, Gruca P, Boyer PA, Mocaer E (2003) Effect of agomelatine in the chronic mild stress model of depression in the rat. Neuropsychopharmacology 28:694–703

Paxinos G, Watson C (1996) The rat brain in stereotaxis coordinates. Academic Press, New York

Pollock GS, Vernon E, Forbes ME, Yan Q, Ma YT, Hsieh T, Robichon R, Frost DO, Johnson JE (2001) Effects of early visual experience and diurnal rhythms on BDNF mRNA and protein levels in the visual system, hippocampus, and cerebellum. J Neurosci 21:3923–3931

Riva MA, Molteni R, Bedogni F, Racagni G, Fumagalli F (2005) Emerging role of the FGF system in psychiatric disorders. Trends Pharmacol Sci 26:228–231

Sheline YI, Gado MH, Kraemer HC (2003) Untreated depression and hippocampal volume loss. Am J Psychiatry 160:1516–1518

Soumier A, Banasr M, Lortet S, Masmejean F, Bernard N, Kerkerian-Le-Goff L, Gabriel C, Millan MJ, Mocaer E, Daszuta A (2009) Mechanisms contributing to the phase-dependent regulation of neurogenesis by the novel antidepressant, agomelatine, in the adult rat hippocampus. Neuropsychopharmacology 34:2390–2403

Turner CA, Akil H, Watson SJ, Evans SJ (2006) The fibroblast growth factor system and mood disorders. Biol Psychiatry 59:1128–1135