Agomelatine, a New Antidepressant, Induces Regional Changes in Hippocampal Neurogenesis

Mounira Banasr, Amélie Soumier, Micheline Hery, Elisabeth Mocaër, and Annie Daszuta

Background: *Antidepressant treatments increase neural plasticity and adult neurogenesis, especially in the hippocampus. Here, we determined the effects of agomelatine (S-20098), a new antidepressant, on various phases of neurogenesis in the dentate gyrus of adult rat.*

Methods: *Animals were injected with agomelatine for different time periods. Immunostaining for bromodeoxyuridine, neuron specific nuclear protein, and glial fibrillary acid protein, as well as for the highly polysialylated form of neuronal cell adhesion molecule and doublecortin, was used to detect changes in cell proliferation, neurogenesis, and survival. Cell death was estimated by terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end labeling and cresyl violet staining.*

Results: *Chronic (3 weeks) but not acute (4 hours) or subchronic (1 week) administration of agomelatine increased cell proliferation and neurogenesis in the ventral dentate gyrus, a region notably implicated in response to emotion, which is consistent with the antidepressant–anxiolytic properties of the drug. Extending agomelatine treatment over several weeks, however, increases survival of newly formed neurons in the entire dentate gyrus. Finally, agomelatine treatment does not affect mature granule cells.*

Conclusions: *This study shows that an antidepressant can affect differentially various stages of neurogenesis in the dorsal and ventral hippocampus. Altogether, these changes lead to a pronounced augmentation in the total number of new granule cells.*

Key Words: Anxiety, ventral hippocampus, proliferation, survival, differentiation, adult rat

Depression is one of the most common psychiatric disorders known to alter pivotal functions, such as mood, cognition, or psychomotor activities. Depression or stress-induced symptoms are now frequently associated with structural changes in the brain and decreases in neuroplasticity. Clinical studies described decreases in hippocampal volume in patients with stress-related major depression (Bremner et al 2000; MacQueen et al 2003; Rajkowska 2000; Sheline 2003) or posttraumatic stress disorder (Sapolsky 2001; Vermetten et al 2003; Wignall et al 2004). Such a reduction in hippocampal volume might be due to glial and neuronal atrophy or loss, which is related in part to increases in corticosteroids and excitatory amino acids, as demonstrated in animal models (Fuchs and Flugge 1998; Lee et al 2002; McEwen 2000); such relationships are still under investigation in humans (Lucassen et al 2001; Vythilingham et al 2004).

The dentate gyrus (DG) of the hippocampal formation is a site of continuous neurogenesis during adult life in mammals, including humans (Eriksson et al 1998). Generated in the subgranular zone (SGZ) of the DG, a portion of newborn cells becomes neurons (Cameron et al 1993; Kuhn et al 1996), are functionally integrated into hippocampal circuits (Song et al 2002; Van Praag et al 2002), and are involved in certain forms of hippocampal learning and memory (Kempermann et al 2004; Shors et al 2001, 2002). Hippocampal adult neurogenesis is influenced by environmental (Kempermann et al 1997) and internal signals, including hormones, neurotransmitters and growth factors (Fuchs and Gould 2000), as well as various pharmacological compounds (Duman et al 2001; Eisch 2002). Importantly, stressful experiences have been shown to diminish the genesis of hippocampal granule cells in a number of mammalian species (Duman 2004a; Gould and Tanapat 1999). Decreases in granule cell neurogenesis and neuronal atrophy of pyramidal neurons can contribute to the hippocampal volume reduction observed after chronic stress (Czeh et al 2001; McEwen 1999). Antidepressant treatments can prevent or reverse some of these structural alterations, as cellular atrophy or death (Lucassen et al 2004; Magarinos et al 1999; McEwen 1999), reduction of hippocampal volume, and decrease in cell proliferation (Czeh et al 2001; Malberg and Duman 2003; van der Hart et al 2002). Beside the actual debate on the functional implication of hippocampal neurogenesis in depression and antidepressant action (Henn and Vollmayr 2004; Sapolsky 2004), the antidepressant effects on neurogenesis promote the current cellular neuroplasticity hypothesis of major depression (Benninghoff et al 2002; Duman 2004b; Duman et al 2001; Eisch 2002; Jacobs et al 2000; Kempermann 2002; Malberg 2004; Malberg and Schechter 2005).

A large number of antidepressant and mood-stabilizing treatments increase the formation of new neurons in the DG of unstressed animals, including serotonin- and norepinephrine-enhancing drugs (Malberg et al 2000; Manev et al 2001), electroconvulsive seizures (Madsen et al 2000; Scott et al 2000), and lithium (Chen et al 2000). The present study was designed to assess the effects of a new antidepressant drug, agomelatine (S-20098), on adult neurogenesis in the rat brain. Agomelatine can resynchronize circadian rhythms in rodents (Tuma et al 2001) and has strong effects in several animal models of depression, such as chronic mild stress, forced swim test, bulbectomy, learned helplessness test, or prenatal stress (Bertaina-Anglade et al 2002; Bourin et al 2004; Morley-Fletcher, unpublished data; Norman et al, unpublished data; Papp et al 2003). In humans, clinical studies demonstrated the efficacy of agomelatine in major depressive disorders (Loo et al 2002a; 2002b; 2003). This antidepressant acts through a unique mechanism of action, as a melatonin agonist and a 5-hydroxytryptamine (5-HT)2C antagonist (Millan et al 2003), and has anxiolytic properties (Millan et al 2005). Although it has a modest antidepressant effect (Papp et al 2003), melatonin has been shown recently to increase hippocampal cell proliferation in maternally separated pups (Kim et al

From the Cell Interactions, Neurodegeneration and Neuroplasticity Unit (MB, AS, MH, AD), Unité Mixte de Recherche 6186, Centre National de la Recherche Scientifique, Marseille; and the International Research Institute Servier (EM), Courbevoie, France.

Address reprint requests to Annie Daszuta, M.D., IC2N, CNRS, Cell Interactions, Neurodegeneration and Neuroplasticity Unit, UMR 6186, 31 Ch. J. Aiguier, Marseille 13009, France; E-mail: daszuta@ibdm.univ-mrs.fr.

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2004). In contrast, blockade of 5-HT2C receptors might have a therapeutic action on anxiety (Martin et al 2002) and does not seem to alter hippocampal neurogenesis (Banasr et al 2004). This last result contrasts with the effect of other 5-HT receptor subtypes and the large amount of literature showing changes in hippocampal cell proliferation after alterations in 5-HT transmission (Banasr et al 2001, 2004; Brezun and Daszuta 1999, 2000a, 2000b; Radley and Jacobs 2002; Rosenbrock et al 2005; Veenema et al 2004) and fluoxetine treatments (Kodama et al 2004; Lee et al 2001; Malberg and Duman 2003; Malberg et al 2000; Manev et al 2001; Namestkova et al 2005; Sairanen et al 2005; Santarelli et al 2003). Consequently, studying agomelatine's effects on adult neurogenesis was of particular interest.

After acute, subchronic, or chronic treatment with agomelatine, bromodeoxyuridine (BrdU), a thymidine analogue that labels dividing cells in S-phase, was administered to assess possible changes in cell proliferation. Effects of agomelatine treatment on neurogenesis were then examined 3 weeks after multiple injections of BrdU. Differentiation of newly formed cells was analyzed by triple labeling for mature neurons with neuron specific nuclear protein (NeuN) and for glial cells with glial fibrillary acid protein (GFAP). Potential changes in the number of cells immunoreactive for doublecortin (DCX) and PSA-NCAM, the highly polysialylated form of neuronal cell adhesion molecule, as markers of newly formed granule cells, were also examined (Brown et al 2003; Seki 2002). This study was completed by showing the effects of agomelatine treatment on the survival of newly formed cells.

Methods and Materials

Animals

Adult 8-week-old male Wistar rats (220–240 g; Iffa Credo, l'Arbresles, France) were group-housed (three per cage) in a temperature-controlled room (21°C) and maintained on a 12-hour light/dark cycle with free access to food and water. This study was carried out in accordance with the French Agriculture and Forestry Ministry (decree 87848, license 01498). There were five to seven rats per group.

Treatment

The dose of agomelatine used in the present study was chosen on the basis of its activity in various animal models of depression and anxiety in the range of 2–50 mg/kg p.o. or IP (Bourin et al., 2004; Millan et al 2005; Papp et al 2003). In the present study, agomelatine (40 mg/kg) was administered IP as a suspension in 1% hydroxyethylcellulose.

Experiment 1: Agomelatine-Induced Changes in Cell Proliferation

To determine whether acute (4 hours), subchronic (1 week), or chronic treatment (3 weeks) affected cell proliferation in the SGZ, systemic injections of agomelatine or vehicle (hydroxyethylcellulose) were performed (Figure 1A). For 1- or 3-week treatments, agomelatine and vehicle were administered every day at 5:00 PM. The next morning, rats received BrdU (200 mg/kg IP) and were perfused 2 hours later. For acute experiments, rats received the same dose of agomelatine at 2:00 PM, BrdU 2 hours later, and they were perfused 2 hours after BrdU.

Experiment 2: Agomelatine-Induced Changes in Neurogenesis and Survival

For determination of long-term induced changes in neurogenesis, rats were treated with agomelatine for 3 weeks and allowed to survive 3 more weeks after the last BrdU administration (group 3W). In this experiment, BrdU (75 mg/kg IP) was injected twice daily (6-hour intervals) during the last 3 days of drug administration (Figure 1B). Respective control animals followed the same protocol with vehicle and BrdU administrations.

A similar protocol was used to assess a putative additive effect of agomelatine on survival, except that animals received the drug over 6 weeks, covering the 3 weeks of differentiation after the last BrdU injection (group 6W) (Figure 1C).

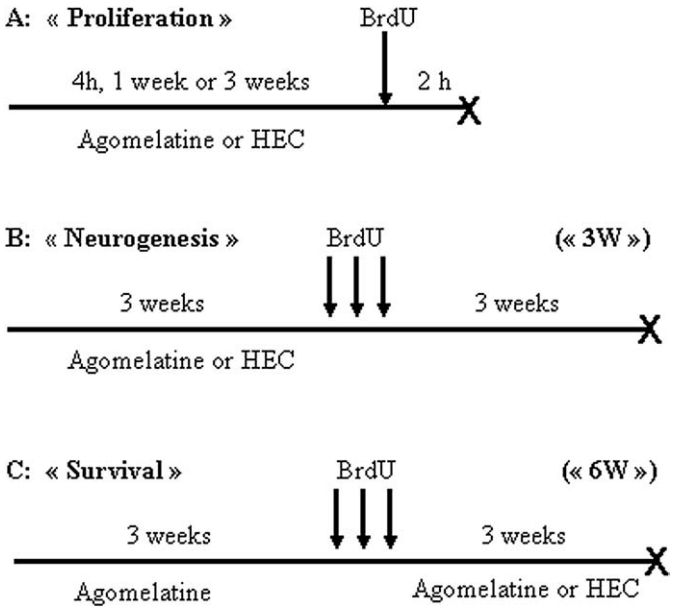

Figure 1. Experimental designs and group assignments. **(A)** Proliferation studies were conducted in rats treated with agomelatine or corresponding solvant (hydroxyethylcellulose [HEC]) for various time periods (4 hours, 1 week, 3 weeks) and perfused 2 hours after bromodeoxyuridine (BrdU) injection (200 mg/kg). **(B)** Neurogenesis study was conducted in rats treated for 3 weeks with agomelatine or HEC, injected with BrdU twice daily (75 mg/kg) at days 19, 20, and 21, and perfused 3 weeks after the last BrdU injection. **(C)** Survival study was conducted in similar groups as for neurogenesis, but agomelatine treatment was continued after BrdU injections.

Tissue Preparation

Rats were deeply anesthetized with chloral hydrate (400 mg/kg IP) and transcardially perfused with 500 mL of cold 4% paraformaldehyde in .1 mol/L phosphate-buffered saline (PBS, pH 7.4). The brains were postfixed overnight at 4°C in the same solution and left for 2 days in PBS containing 30% sucrose for cryoprotection. Then, 40-μm-thick frontal and serial sections of DG and subventricular zone (SVZ) were obtained with a cryostat. Sections were stored at −20°C in cryoprotectant solution until staining (1/3 vol/vol glycerol, 1/3 vol/vol ethylene glycol, 1/3 vol/vol .1 mol/L phosphate buffer, pH 7.4).

Immunohistochemistry

Newborn cells were detected by peroxidase immunostaining for BrdU, a marker of cell division (Cooper-Kuhn and Kuhn 2002). Free-floating sections were first rinsed in .1 mol/L PBS and incubated for 15 min in 1% H_2O_2 and 10% fetal calf serum for 2 hours as the blocking buffer. Then, sections were incubated in 2 N HCL and .5% Triton-X100 in PBS (30 min, 37°C) for deoxyribonucleic acid hydrolysis. Sections were rinsed in .1 mol/L sodium tetraborate buffer, pH 8.5, then incubated (overnight + 24 hours) with the primary antibody (monoclonal mouse immu-