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REVIEW

Agomelatine: mechanism of action and pharmacological profile in relation to antidepressant properties

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Agomelatine behaves both as a potent agonist at melatonin MT_1 and MT_2 receptors and as a neutral antagonist at 5-HT_{2C} receptors. Accumulating evidence in a broad range of experimental procedures supports the notion that the psychotropic effects of agomelatine are due to the synergy between its melatonergic and 5-hydroxytryptaminergic effects. The recent demonstration of the existence of heteromeric complexes of MT_1 and MT_2 with 5-HT_{2C} receptors at the cellular level may explain how these two properties of agomelatine translate into a synergistic action that, for example, leads to increases in hippocampal proliferation, maturation and survival through modulation of multiple cellular pathways (increase in trophic factors, synaptic remodelling, glutamate signalling) and key targets (early genes, kinases). The present review focuses on the pharmacological properties of this novel antidepressant. Its mechanism of action, strikingly different from that of conventional classes of antidepressants, opens perspectives towards a better understanding of the physiopathological bases underlying depression.

Abbreviations

Arc, activity-regulated cytoskeleton-associated protein; BDNF, brain-derived neurotrophic factor; *CLOCK*, circadian locomotor cycles kaput; CREB, cAMP-responsive element binding protein; DA, dopamine; DCX, doublecortin; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; DRN, dorsal raphé nuclei; DSPS, delayed sleep-phase syndrome; FST, forced swim test; GR-i, glucocorticoid receptor impaired; GSK, glycogen synthase kinase; H, helpless; HPA, hypothalamic pituitary adrenal; LC, locus coeruleus; L/D, light-dark; mGlu, metabotropic glutamate; MT, melatonin; NA, noradrenaline; NH, non-helpless; PTSD, post-traumatic stress disorder; REM, rapid eye movement; SCN, suprachiasmatic nucleus; SWS, slow wave sleep; TST, tail suspension test; vGLUT, vesicular glutamate transporters; VTA, ventral tegmental area

Introduction

Strong links exist between abnormal patterns of circadian rhythms and some of the most characteristic symptoms of affective disorders, including unipolar and bipolar depression (Wirz-Justice and Van den Hoofdakker, 1999; McClung, 2007; Turek, 2007). These symptoms include delayed sleep onset, non-restful sleep, early-morning wakening, blunting or reversal of the normal morning peaks in subjective energy, mood or alertness, and daytime fatigue (Germain and Kupfer, 2008). Hence, the human circadian system holds possible important clues regarding the aetiology and the treatment of affective disorders. Actually, circadian patterns of gene expression are disrupted in major depressive disorders (Li *et al.*, 2013).

Therefore, an innovative approach to improve the treatment of depression in patients with abnormal circadian rhythmicity may include the administration of circadian phase-shifting agents. The goal of such treatment is not only to restore sleep–wake patterns (Guardiola-Lemaitre and Quera-Salva, 2011), but also to resynchronize the circadian system and its link with the external environment. This way of thinking lead to the discovery of agomelatine (Adam *et al.*, 1992; Yous *et al.*, 1992), a compound that combines melatonin receptor (see Alexander *et al.*, 2013a) agonism with 5-HT_{2C} receptor antagonism (Delagrange and Guardiola-Lemaitre, 1997).

The mode of action of agomelatine is obviously different from that of other available antidepressants. The psychotropic effects of agomelatine are caused by the synergy between the melatonergic and 5-HT_{2C} receptor properties of the compound (Racagni *et al.*, 2011). Both animal experimental and human clinical data establish that this innovative pharmacology translates into a clinical benefit (Kennedy *et al.*, 2008; Kennedy and Rizvi, 2010; Martinotti *et al.*, 2012). Critical analyses of the available clinical studies with agomelatine have appeared elsewhere (de Bodinat *et al.*, 2010; Kennedy and Rizvi, 2010; Hickie and Rogers, 2011). Likewise, the history of the discovery and development of agomelatine have been summarized elsewhere (de Bodinat *et al.*, 2010). In the present review, we aim to provide a comprehensive inventory of the current knowledge of the cellular actions and the pharmacological properties of agomelatine in animal models, which predict clinical antidepressant efficacy.

Binding properties

Using an enzymatic assay to measure cAMP (as no cloned melatonin receptors were available at that time), it was first demonstrated that, similar to melatonin, micromolar concentrations of agomelatine inhibit the *in vitro* production of cAMP by cells of the pars tuberalis of the sheep. In addition, agomelatine completely displaced 2-iodo-melatonin from its main binding sites in the pars tuberalis (at 10^{-8}M) and in the suprachiasmatic nucleus (SCN) of the hypothalamus (at 10^{-10}M) (Bonnefond *et al.*, 1993). Binding assays with transfected cells and native tissues confirmed that agomelatine has a high affinity for human melatonin MT_1 and MT_2 receptors (K_i ~10^{-10}M) and behaves *in vitro* as a melatonergic agonist (Audinot *et al.*, 2003).

That the mechanism of action of agomelatine differs from that of other classes of antidepressants is exemplified by the finding that it has no affinity (K_i > 10 μM) for most screened receptors, including adenosine, adrenoceptors, dopamine (DA), GABA, muscarinic, nicotinic, histamine, excitatory amino acid, benzodiazepine and sigma receptors, as well as sodium, potassium and calcium channels. Similarly, most 5-HT receptors are not recognized by the compound, which has only some affinity for the 5-HT_2 receptor family. Indeed, agomelatine binds with 5-HT_{2C} receptor (pK_i = 6.2) and interacts also with cloned, human 5-HT_{2B} receptors (pK_i = 6.6), but shows negligible affinity at 5-HT_{2A} receptors (pK_i < 6) (Millan *et al.*, 2003). That agomelatine acts as an antagonist at 5-HT_{2C} and HT_{2B} receptor subtypes has been established in transfected cells expressing human 5-HT_{2C} receptors, in which the compound antagonizes both the 5-HT-induced specific binding of [^{35}S]-GTP-γ-S to G-proteins specifically coupled to the receptors, and the 5-HT-induced activation of PLC.

Agomelatine behaved as an antagonist at human 5-HT_{2B} receptors in an assay of [^{3}H]-phosphatidylinositol depletion, but the functional significance of this activity to the central action of the compound remains unclear. Several findings suggest that 5-HT_{2B} receptors play a functional role in the periphery and in the brain (Brea *et al.*, 2010) and predict that no noxious and perhaps even beneficial effects of agomelatine may be expected in humans based on 5-HT_{2B} receptor antagonist properties. For example, 5-HT_{2B} receptor antagonists can reduce arterial blood pressure in hypertensive rats (Watts and Fink, 1999), prevent cardiac hypertrophy in mice (Jaffre *et al.*, 2004; Monassier *et al.*, 2008) and improve neuropathic, visceral pain and hyperalgesia in rodents (Aira *et al.*, 2010; O'Mahony *et al.*, 2010; Lin *et al.*, 2011; Cervantes-Duran *et al.*, 2012). Agomelatine does not interact with classical targets of antidepressant drugs, namely MAO A and B and the transporters responsible for the re-uptake of 5-HT, NA or DA (de Bodinat *et al.*, 2010). In line with these data, acute or chronic treatments with agomelatine (10 or 50 mg·kg^{-1}·day^{-1} i.p. for up to 3 weeks) did not affect the extracellular levels of 5-HT in the frontal cortex of adult rats (Millan *et al.*, 2003). Moreover, chronic agomelatine administration does not down-regulate 5-HT_{1A} receptors (Hanoun *et al.*, 2004) and does not affect extracellular levels of 5-HT (Millan *et al.*, 2005). Actually, owing to the absence of facilitatory influence of agomelatine on central and/or peripheral actions of 5-HT, it does not cause, the major adverse effects of the conventional antidepressants [e.g. gastrointestinal disorders, sleep disturbances (e.g. rapid eye movement – REM – sleep inhibition) and sexual dysfunctions (de Bodinat *et al.*, 2010)].

Although the affinity of agomelatine at 5-HT_{2C} receptors is about 1000-fold lower than that at MT_1 and MT_2 receptors, the functional 5-HT_{2C} antagonistic property of agomelatine has been established *in vivo* at antidepressant doses (10–50 mg·kg^{-1}) (Chagraoui *et al.*, 2003). A MRI study in rats shows that agomelatine in the same dose range (10–40 mg·kg^{-1}) blocks central 5-HT_{2C} receptors *in vivo* within a broad range of corticolimbic structures, including the hippocampus (Linnik *et al.*, 2009).

The 5-HT_{2C} receptors are the only known GPCRs to be subjected to RNA editing, a mechanism that can generate multiple variants of a particular gene, thereby producing 5-HT_{2C} receptor isoforms with various properties (i.e. affinity, coupling and constitutive activity) (Werry *et al.*, 2008). In this context, the interaction of agomelatine with this receptor subtype may be more complex than initially thought as the compound may be a more potent antagonist for certain 5-HT_{2C} isoforms. Thus, while agomelatine behaves as an antagonist at human edited 5-HT_{2C} receptors, it acts as a *neutral* antagonist at unedited and constitutively active 5-HT_{2C} receptors (Millan *et al.*, 2011b). The interaction of agomelatine has been examined with unedited human (h) 5-$HT_{2C(INI)}$ receptors expressed in HEK-293 cells, and with native, 5-HT_{2C} receptors of mouse cortical neurons, two populations of receptors known to display constitutive activity. Agomelatine behaves as a neutral antagonist at constitutively active h5-$HT_{2C(INI)}$ receptors and native, cerebral 5-HT_{2C} recep-

tors. In fact, agomelatine does not affect activity of GPCRs, but rather normalizes signalling at 5-HT_{2C} sites by blocking the actions both of agonists and of inverse agonists, thereby returning activity to baseline value rather than suppressing it (Millan *et al.*, 2011b).

It is difficult to extrapolate from cellular findings, but inasmuch as 5-HT_{2C} receptors in the brain are constitutively active, the neutral antagonist profile of agomelatine may be important for at least two reasons. Firstly, it may explain why agomelatine does not markedly accelerate subcortical versus fronto-cortical dopaminergic transmission. Indeed, agomelatine can increase the extracellular levels of dopamine and NA in the frontal cortex, whereas no change in dopamine level is seen in the nucleus accumbens or the striatum (Millan *et al.*, 2003). Accordingly, the acute administration of agomelatine induces a 50% increase in the firing rate of noradrenergic neurons in the locus coeruleus (LC), no changes in the firing pattern of dopaminergic neurons in the ventral tegmental area (VTA) but an increase in spontaneously active cells, and no effect on 5-hydroxytryptaminergic firing activity in the dorsal raphé nuclei (DRN). The agomelatine-induced increase in the number of spontaneously active dopaminergic neurons is, at least in part, mediated by melatonin receptors as it is antagonized by a single administration of the MT_1/MT_2 antagonist S 22153 (Chenu *et al.*, 2013). These effects are in accord with the neutral antagonist property of agomelatine at the 5-HT_{2C} receptor subtype as 5-HT_{2C} receptors that exert an inhibitory influence on mesolimbic and nigro-striatal dopaminergic pathways are constitutively active (Navailles *et al.*, 2006; Aloyo *et al.*, 2009).

Secondly, the neutral antagonist properties of agomelatine are presumably related to its clinical profile with few side effects and no withdrawal syndrome after abrupt discontinuation (Montgomery *et al.*, 2004). Indeed, long-term treatment with a neutral antagonist such as agomelatine does not increase cell surface expression of 5-HT_{2C} receptors (Millan *et al.*, 2011a). Hence, when agomelatine treatment is discontinued, 5-HT will re-access a normal level of 5-HT_{2C} receptors without the negative consequences linked to changes in 5-HT_{2C} receptor density (Chanrion *et al.*, 2008; Aloyo *et al.*, 2009).

The long-term (14 days) administration of agomelatine also enhances the number of spontaneously active neurons and the bursting activity of dopaminergic neurons in the VTA, two effects probably obtained, at least in part, through the activation of melatonin receptors as they are antagonized by S 22153. Further, the firing rate of 5-hydroxytryptaminergic neurons in the DRN is indirectly increased through a DA D_2 receptor-dependent mechanism, while the firing activity of noradrenergic neurons in the LC remains at the control level, perhaps because of the enhanced negative feedback exerted by the up-regulated 5-HT neurotransmission (Chenu *et al.*, 2013).

Although a synergistic mechanism involving the combination of MT_1 and MT_2 melatonin receptor activation and 5-HT_{2C} receptor blockade can explain the molecular, cellular and behavioural actions of agomelatine (Racagni *et al.*, 2011), the precise mechanism underlying this 'crosstalk' between the two receptors remains unknown. Experiments evaluating in HEK293T cells the potential existence of heterodimeric complexes of hMT_1 and hMT_2 receptors with non-edited h5-$HT_{2C(INI)}$ receptors has provided some details about this interplay (Kamal *et al.*, 2010). Co-immunoprecipitation and bioluminescence resonance energy transfer assays demonstrated that 5-HT_{2C} receptors preferentially form heterodimers with MT_2 receptors, but also form, with lower propensity, 5-HT_{2C} homomers or heteromers with MT_1 receptors. The formation of MT_2-5-HT_{2CINI} and MT_1-5-HT_{2CINI} heterodimers in HEK293T cells caused alterations in coupling to ß-arrestin and G_q/ PLC. Basal and maximal 5–HT-activated stimulation of PLC *via* 5-HT_{2C} receptors was increased in the presence of MT_2 and, less markedly, of MT_1 receptors. Melatonin enhanced PLC activation *via* a *Pertussis* toxin-insensitive Gαq in cells co-expressing MT_2 and 5-HT_{2CINI} receptors, suggesting heterodimer transactivation (Kamal *et al.*, 2010). If formation of such heteromers were to occur *in vivo*, this could lead to a synergistic 'crosstalk' between these receptors. Hence, these results at the cellular level have potentially important implications for the understanding of how the two pharmacological properties of agomelatine may translate into a synergistic action.

Effects on circadian rhythms

Agomelatine is unique among antidepressants in modulating circadian rhythms. Thus, agomelatine dose-dependently inhibits the firing of cells in the SCN (the endogeneous master clock) in Syrian hamsters (ED_{50} = 0.91 mg·kg^{-1}, i.p.) (Ying *et al.*, 1996). Chronic agomelatine (given i.p.. or iontophoretically) also does not alter either the sensitivity or the *in vitro* response of SCN cells, demonstrating that there is no desensitization of melatonin receptors after repeated administration of the drug (Ying *et al.*, 1998).

A useful approach to examine whether or not a pharmacological agent can influence the circadian clock is to determine if its acute administration induces a permanent phase shift of the rhythms driven by the clock under constant environmental conditions. The chronobiotic effect of agomelatine was evaluated using the validated model of free-running conditions. The latter are obtained by putting the animals in total darkness so that circadian rhythms are not synchronized by light, thereby allowing free-running of the endogenous rhythms of the biological clock – the SCN. In animals in which the period of endogenous circadian rhythms is greater (rat, hamster) or shorter (mice) than 24 h, circadian rhythms are delayed or advanced every day respectively. Under these conditions, agomelatine synchronizes the circadian rhythm to 24 h by phase advancing circadian rhythms every day. In the rat, this effect is (i) dose-dependent (ED_{50} = 5 mg·kg^{-1} p.o.); and (ii) concentration-dependent, with a correlation between exposure to agomelatine and re-entrainment of circadian rhythms (Martinet *et al.*, 1996).

The entrainment ability of agomelatine is related to a direct stimulating effect of the drug on receptors located in the SCN. Indeed, agomelatine is unable to resynchronize circadian rhythms when the SCN is lesioned. By contrast, pinealectomy has no influence on the resynchronization of circadian rhythms by either agomelatine or melatonin (Redman and Francis, 1998; Pitrosky *et al.*, 1999).

Prolonged s.c. infusion of agomelatine was administered to test the effect of variable duration of exposure to the drug on resynchronization of the circadian rhythm. A pulse effect

(1 h infusion) of agomelatine was sufficient for resynchronizing and as effective as an 8 h infusion. Agomelatine was fully effective after 1 or 8 h s.c. infusion while efficiency decreased with a 16 h infusion, which re-entrained less than half of the animals (Pitrosky *et al.*, 1999). That a short time of exposure to agomelatine is sufficient for resynchronizing circadian rhythms under free-running conditions represents an important finding that explains why the compound resynchronizes circadian rhythms in humans despite a short half-life of around 2 h.

After an 8 h phase-advance of the light–dark (L/D) cycle, agomelatine (1 and 3 mg·kg^{-1}) administered s.c. to Long-Evans rats at the beginning of darkness for 14 days induces a phase-advance and a gradual shift of activity to the onset of darkness similar to that induced by melatonin (Redman *et al.*, 1995). Following a 5 h phase-advance of the 12:12 L/D cycle in the same strain, agomelatine (1 mg·kg^{-1}) phase shifts the free-running rhythms and reduces the number of days needed to re-entrain the animals when administered at the time of the post-, and not at the time of the pre-shift dark onset (Redman and Francis, 1998). Interestingly, the resynchronizing activity of agomelatine has been demonstrated not only in nocturnal, but also in diurnal rodents (Van Reeth *et al.*, 1998).

Re-entrainment in rodents is mediated by a phase-advance of circadian rhythms within a specific therapeutic window (between circadian time 9 and 11), and by restoration of the normal circadian rhythm pattern (Van Reeth *et al.*, 1997). The latter property of agomelatine has also been demonstrated in a model of trypanosome-infected rats with fragmented and disrupted rhythms (Grassi-Zucconi *et al.*, 1996).

In the delayed sleep-phase syndrome model, when free-running rats return to conventional 12:12 L/D cycle, in some of them the onset of activity lags behind that of darkness by about 4 h (negative phase angle difference). In these animals, agomelatine (1, 3 mg·kg^{-1}, s.c.) administered chronically at the beginning of darkness advances locomotor activity and abolishes in 9 days the negative phase shift (Armstrong *et al.*, 1993). In addition to an advance of rhythm onset, agomelatine is able to increase the amplitude of circadian rhythms, such as the daily rhythms in melatonin secretion and core temperature (Castanho *et al.*, 2014).

Agomelatine also influences the sleep/wake cycle architecture in control animals, as demonstrated by the enhanced duration of REM and slow wave sleep (SWS) for 3 h after acute oral administration of the drug (10 and 40 mg·kg^{-1}) shortly before the dark phase of the L/D cycle in rats (Descamps *et al.*, 2009). The same modifications are observed during the following night without additional treatment with agomelatine. Moreover, the compound is without effects when given at light onset. This chronobiotic profile of agomelatine is underlined by the lack of alterations in latencies to SWS and REM sleep and the preservation of the spectral components of the electroencephalogram (EEG) (Cajochen *et al.*, 1997; Quera Salva *et al.*, 2007; Descamps *et al.*, 2009). Indeed, melatonin and the melatonin agonist ramelteon exerted different effects on the parameters mentioned earlier and on power band spectra of the EEG, possibly reflecting the absence of antagonistic action at 5-HT$_{2C}$ receptors. Actually, underscoring the importance of 5-HT$_{2C}$ receptor blockade for agomelatine, the 5-HT$_{2C}$ antagonist S 32006 (10 mg·kg^{-1}), administered i.p. at the onset of the dark phase mimicked the increased SWS provoked by agomelatine, yet diminished REM sleep (Descamps *et al.*, 2009).

Chronic treatment of rats with agomelatine (2000 ppm in the diet) corrects the circadian rhythm abnormalities of motor activity and sleep/wake cycle (e.g. reduced duration of SWS, increased duration of REM sleep) induced by prenatal restraint stress, a model of depression; these effects are blocked by the MT$_1$/MT$_2$ receptor antagonist S22153 (Mairesse *et al.*, 2013).

Ageing has been associated with abnormal circadian time keeping, including changes in rhythms amplitude and period, as well as changes in synchronization of those rhythms by the L/D cycle (Turek *et al.*, 2001). Age-related changes in the mammalian circadian system may be associated with a decline in circulating melatonin levels. In old hamsters showing low circulating levels of endogenous melatonin, agomelatine restores the sensitivity of the circadian clock to its synchronizers (Weibel *et al.*, 2000) and accelerates by approximately 25% the resynchronization of their circadian activity rhythm after an abrupt shift in the L/D cycle (Van Reeth *et al.*, 2001). Chronic administration of agomelatine significantly increases the phase-advancing effects of a light pulse, and induces a lengthening of the free-running period of the activity rhythm in those animals (Van Reeth *et al.*, 2001).

The findings mentioned earlier have been confirmed in young, healthy volunteers receiving agomelatine orally in a wide dose range (5–100 mg). Thus, these studies demonstrated persistent phase-advances of circadian rhythms, apparent even the day following agomelatine administration. Agomelatine produced a phase-advance in the circadian changes in temperature and melatonin levels and advanced the timing of the daily decrease in heart rate (Krauchi *et al.*, 1997). The termination of the sleep period and REM sleep propensity were phase-advanced, without effect on other sleep variables (Cajochen *et al.*, 1997). Phase shifting effects have also been reported in healthy elderly men, with advances in the timing of the temperature and cortisol rhythms induced by agomelatine, again with no effect on sleep variables (Leproult *et al.*, 2005). In depressed patients, agomelatine enhances SWS without modifying REM sleep, and advances the time of sleep onset and that of minimal heart rate (Quera Salva *et al.*, 2007). In line with its mode of action, a treatment with agomelatine improved the amplitude of the circadian rest–activity/sleep–wake cycle and also decreased the symptoms of depression and anxiety (Kasper *et al.*, 2010).

Antidepressant effects in animal models

Resynchronization of biological rhythms may be a therapeutically relevant property for an antidepressant agent as depression involves a disruption of normal circadian rhythmicity, including an abnormal phase relationship of various circadian rhythms with the external environment (Kronfeld-Schor and Einat, 2012). With the limits of such a model in mind (multiple behavioural abnormalities, com-

pensatory mechanisms that alter the adult animal's behavioural response) mice missing MT_1 receptors exhibit abnormalities including melatonin-mediated phase shift deficits (Dubocovich *et al.*, 2005) and increases in depressive-like behaviour demonstrated in the forced swim test (FST) (Weil *et al.*, 2006). Unlike agomelatine, melatonin is not effective in depressed patients (Dubocovich, 2006; Hickie and Rogers, 2011). Therefore, the fact that agomelatine possesses 5-HT_{2C} receptor antagonist properties must confer a therapeutic advantage compared with a pure melatonergic agent for treating depression.

The antidepressant effect of agomelatine has been illustrated in several well-established rodent models reflecting core clinical features of depression, including the FST (Bourin *et al.*, 2004), learned helplessness (Bertaina-Anglade *et al.*, 2006), chronic mild stress (Papp *et al.*, 2003), bulbectomy (Norman *et al.*, 2012), psychosocial stress (Schmelting *et al.*, 2014), prenatal restraint stress (Morley-Fletcher *et al.*, 2011; Marrocco *et al.*, 2014) and corticosterone-induced depression/anxiety (Rainer *et al.*, 2012). Effects of agomelatine were principally apparent after chronic treatment. Indeed, acute administration of agomelatine has only variable, weak and inconsistent effects in FST procedures in rodents (M.J. Millan, unpubl. obs.).

The antidepressant effects of agomelatine have been also demonstrated in two genetic models of depression. Thus, in transgenic glucocorticoid receptor-impaired (GR-i) mice, GR receptors are down-regulated, thereby making GR-mediated feedback control of hypothalamic–pituitary–adrenal (HPA) axis activity deficient as in depressed patients (Pepin *et al.*, 1992a,b). Accordingly, GR-i mice exhibit numerous behavioural and biological abnormalities that can be suppressed by evening treatment with agomelatine (10 mg·kg^{-1}, i.p. for 21 days). In addition, chronic agomelatine (for 21 days) reverses the behavioural and neuroplastic changes associated with HPA axis alterations in these GR-i mice (Barden *et al.*, 2005; Paizanis *et al.*, 2010).

Another genetic model of depression is based on a selective breeding of Swiss albino CD1 mice with strikingly different responses in the tail suspension test (TST). The lines of mice were named H/Rouen and NH/Rouen for mice displaying *helpless* (H) and *non-helpless* (NH) phenotypes in the TST respectively (El Yacoubi *et al.*, 2003). The H/Rouen line shows great stability in terms of immobility scores, anhedonia and altered 5-hydroxytryptaminergic transmission. Chronic administration of agomelatine (10–50 mg·kg^{-1}, i.p. for 3 weeks) significantly reduces immobility of H/Rouen mice in the TST and increases their sucrose preference as compared with vehicle-treated animals (El Yacoubi *et al.*, 2011).

Agomelatine has proven resynchronizing properties in animal models of depression. It is effective in a chronic psychosocial stress model in tree shrew (*Tupaia belangeri*) (Fuchs, 2005). Animals show profound disturbances in their circadian rhythms when exposed to the social stress procedure. In this model, chronic agomelatine antagonizes the effects of stress on circadian changes in body temperature while melatonin or fluoxetine do not (Schmelting *et al.*, 2014). Agomelatine also accelerates the re-establishment of normal circadian rhythms of temperature and activity in genetically modified GR-i mice (Barden *et al.*, 2005). Chronic administration of agomelatine increases the amplitude of circadian rhythm activity in mice with corticosterone-induced depression/anxiety, an effect that is not observed after the chronic administration of fluoxetine (Rainer *et al.*, 2012). Finally in rats, chronic treatment with agomelatine also corrects the prenatal restraint stress-induced abnormalities in the circadian rhythm of motor activity (Mairesse *et al.*, 2013).

A number of findings obtained in some of the animal models mentioned earlier strongly suggest that the antidepressant-like effect of agomelatine relies on a synergistic action at both melatonin and 5-HT_{2C} receptors (see Table 1). Thus, in the learned helplessness rat model, neither the daily administration of the 5-HT_{2C} receptor antagonist SB242084 (up to 20 mg·kg^{-1}, i.p.) nor melatonin (2, 10 and 50 mg·kg^{-1} given orally using the same schedule as agomelatine) provide an equivalent reduction in the number of escape failures than agomelatine (Bertaina-Anglade *et al.*, 2006). In this model, the response to agomelatine [given in the evening at a time when its chronobiotic effects are fully expressed (Van Reeth *et al.*, 1997)] are prevented by pretreatment with the MT_1/MT_2 receptor antagonist, S 22153 (20 mg·kg^{-1}, i.p.). In the chronic mild stress model, the time of administration of agomelatine [at the beginning of the light period, or in the late evening, just before the start of the dark period] has no influence on its efficacy (Papp *et al.*, 2003). In fact, the neurobiological mechanism underlying the action of agomelatine at the beginning of the light period is not melatonin-like and cannot be reversed by inhibiting the melatonin receptors with S 22153 (20 mg·kg^{-1} i.p.) (Papp *et al.*, 2003). This morning action of agomelatine is more probably based on the 5-HT_{2C} antagonist properties of the drug (Dekeyne *et al.*, 2008). In the chronic psychosocial stress model in tree shrew, the actions of agomelatine cannot be mimicked by the administration of either melatonin or a 5 HT_{2C} receptor antagonist (Schmelting *et al.*, 2014), which also supports the idea that the antidepressant-like effect of agomelatine in this model of depression probably relies on a synergistic action at both receptors. In bulbectomized rats, the action of agomelatine needs its 5-HT_{2C} antagonist property because the blockade of this receptor subtype by S 32006 (0.16–10 mg·kg^{-1}) dose-dependently attenuated hyperactivity. As the maximal effect of the 5-HT_{2C} antagonist is less marked than that of agomelatine (at 10 and 50 mg·kg^{-1}), a possible synergy with melatonin agonist properties cannot be excluded (Norman *et al.*, 2012).

Anxiolytic effects in animal models

Agomelatine possesses anxiolytic properties in several animal models in the same dose range as its antidepressant effect (Table 2). Thus, the anxiolytic-like activity of the compound is comparable with that of the benzodiazepine clorazepate in the plus maze test, the social interaction and Vogel tests (Millan *et al.*, 2005). In the elevated plus maze test, when experiments are carried out during the light period of the diurnal cycle, agomelatine (from 40 mg·kg^{-1}, i.p.) dose-dependently increases the percentage of entries into open arms of the plus maze. In the Vogel test, the morning administration of agomelatine elicits a potent effect. The anxiolytic-like activity of agomelatine in the three tests is comparable with that of the selective 5-HT_{2C} receptor antagonist,

Table 1

Antidepressant activity of agomelatine. Potential synergy between melatonergic agonist and 5-HT_{2C} receptor antagonist properties

Model	Agomelatine	Melatonin	5-HT_{2C} antagonists	Melatonin antagonist#
Despair test(1)	+	–	–	Not tested
Learned helplessness(2)	+	–	–	Antagonism
Bulbectomy(3)	+	–	+*	Not tested
Chronic mild stress(4)	+	+	+*	Antagonism†
Transgenic model (GR-i mice)(5)	+	+	?	Not tested
Psychosocial stress(6)	+	–	+*	Not tested
Isolated aggressive mice	+	±*	+	Not tested
Marble burying test	+	±*	+	Not tested
	Full spectrum of antidepressant activity	Melatonergic effect not sufficient	5-HT_{2C} antagonism not sufficient	

+effect; –no effect; #S22153.
(1) (Bourin *et al.*, 2004); (2) (Bertaina-Anglade *et al.*, 2006); (3) (Norman *et al.*, 2012); (4) (Papp *et al.*, 2003); (5) (Barden *et al.*, 2005; Paizanis *et al.*, 2010); (6) (Schmelting *et al.*, 2014).
*Less effective than agomelatine.
**High doses (120–160 $mg \cdot kg^{-1}$).
†Agomelatine given in the evening.

Table 2

Anxiolytic activity of agomelatine. Potential synergy between melatonergic agonist and 5-HT_{2C} antagonist properties

Model	Agomelatine	Melatonin	5-HT_{2C} antagonists	Melatonin antagonist#
Elevated plus maze(1)	+	+	–	Antagonism
Social defeat(2)	+	+	?	Not tested
Geller Seifter conflict	+	–	+	Not tested
Vogel conflict(3)	+	–	+	No antagonism
Social interaction(3)	+	–	+	No antagonism
Predator scent stress (PTSD model)(4)	+	?	?	Not tested
Predator scent stress(5)	+	?	?	Not tested
Fear conditioning(6)	+	–	–	Antagonism
	Full spectrum of anxiolytic activity	Melatonergic effect not sufficient	5-HT_{2C} antagonism not sufficient	

+effect ; –no effect; #S22153.
(1) (Papp *et al.*, 2006); (2) (Tuma *et al.*, 2005); (3) (Millan *et al.*, 2005); (4) (Koresh *et al.*, 2012); (5) (Conboy *et al.*, 2009); (6) (Diaz-Mataix L *et al.*, 2012).

SB243,213 (Millan *et al.*, 2005), whereas melatonin is ineffective in all these models. The anxiolytic action of agomelatine in the elevated plus maze depends on a synergistic blockade of 5-HT_{2C} receptors and activation of MT_1/MT_2 receptors. This conclusion is based on the observations that, firstly, the anxiolytic action does not depend on the time of agomelatine administration (i.e. morning vs. evening), and secondly, is inhibited by the MT_1/MT_2 receptor antagonist S 22153 when the drug is administered in the evening but not in the morning (Papp *et al.*, 2006). The chronic administration of agomelatine (40–50 $mg \cdot kg^{-1}$, i.p.) for 3 weeks can reverse increased anxiety in models of depression such as prenatal restraint stress (Morley-Fletcher *et al.*, 2011; Marrocco *et al.*, 2014) and corticosterone-treated mice (Rainer *et al.*, 2012).

In the elevated plus maze test, rats receiving agomelatine (50 $mg \cdot kg^{-1}$, i.p. for three days) displayed significantly less anxiety-like behaviours following the predator scent stress exposure, a model of post-traumatic stress disorder (PTSD) (Koresh *et al.*, 2012). In this model, the anxiolytic-like effect of agomelatine was accompanied by a normalization of the

expression of circadian-related genes (Koresh *et al.*, 2012), which again suggests a major role for the resynchronizing action of agomelatine.

Agomelatine also reduces the duration of vocalization by rats re-exposed to an environment associated with an aversive stimulus (conditioned ultrasonic vocalization test) at both low (10–75 mg·kg^{-1}, i.p.) and high doses (100 and 125 mg·kg^{-1}, i.p.), regardless of the time of administration (Papp *et al.*, 2006). The social defeat test allows detection of antidepressant-like effects of chronically administered drugs. However, when animals are confronted to an aggressive dominant congener on a single occasion only, it is usually regarded as a model of acute stress and is useful to detect anxiolytic-like effects of compounds. Under the latter conditions, agomelatine mimics the anxiolytic-like actions of melatonin against acute social defeat in Wistar rats. The effect of agomelatine is abolished by lesions of the SCN (Tuma *et al.*, 2005), which supports a role for both melatonin and 5-HT$_{2C}$ receptors, highly expressed in this brain area. Indeed, 5-HT$_{2C}$ receptors may be recruited for this anxiolytic action since similar effects are obtained in the same paradigms when using experimental conditions of pharmacological or genetic inactivation of 5-HT$_{2C}$ receptors (Millan *et al.*, 2005; Heisler *et al.*, 2007; Dekeyne *et al.*, 2008).

Procognitive effects in animal models

Depressive disorders may affect the ability to think, concentrate, make decisions, formulate ideas, reason and remember. Less clear are the nature and extent of such disturbances, as well as their specificity to affective illness (Marazziti *et al.*, 2010). As a matter of fact, depressive disorders are associated with impairments in different cognitive domains such as executive function, working memory and attention (Baune *et al.*, 2010; Hammar *et al.*, 2010).

Because dopaminergic and noradrenergic mechanisms in the frontal cortex modulate cognitive and attentional performances, mood and motor behaviours, all of which are deeply perturbed in depressive states, it can be reasonably assumed that the stimulant effects of agomelatine on these monoaminergic systems (Millan *et al.*, 2003) contribute not only to its antidepressant action, but also to improved neurocognitive functions, especially memory, attention and problem-solving. The chronobiotic feature of agomelatine could also be relevant in this aspect as a link may exist between circadian disruption and impairments in learning and memory tasks, especially in the area of memory recall (Fekete *et al.*, 1985; Devan *et al.*, 2001; Craig and McDonald, 2008).

Actually, memory-facilitating effects of agomelatine have been demonstrated experimentally, in addition to its antidepressant effects: studies using a radial-arm water maze showed that the chronic administration of agomelatine (10 mg·kg^{-1}, i.p. for 22 days) reversed spatial memory impairment in the predator-stressed rat (Conboy *et al.*, 2009). This promnesiant effect of agomelatine treatment was associated with an increase in hippocampal levels of the neural cell adhesion molecule, known to be involved in memory consolidation (Conboy *et al.*, 2009).

A cognitive-enhancing property of agomelatine has also been demonstrated in the rat two-trial object recognition model (Bertaina-Anglade *et al.*, 2011; Ladurelle *et al.*, 2012). A single i.p. administration of agomelatine at antidepressant doses (2.5, 10 or 40 mg·kg^{-1}), either in the morning or in the evening, enhances performances in this paradigm (Bertaina-Anglade *et al.*, 2011). Positive acute effects on memory performance are also seen upon administering either melatonin at equivalent doses or upon treatment with the 5-HT$_{2C}$ receptor antagonist SB 242,084 (0.63, 2.5 or 10 mg·kg^{-1}). In this paradigm, chronic i.p. administration of agomelatine (40 mg·kg^{-1}·day^{-1}, for 20 days) is likewise active (Ladurelle *et al.*, 2012), indicative of a sustained influence upon cognitive performance. Finally, chronic treatment with agomelatine reversed the disruption of recognition in the prenatal stress model of depression in rats (Marrocco *et al.*, 2014).

Mechanistic issues for the properties of agomelatine

From melatonin and 5–HT$_{2C}$ receptor-based mechanisms . . .

While it is not surprising that the chronobiotic effects of agomelatine involve the agonist property of the drug at melatonin receptors, the effect of agomelatine in the resynchronization of circadian rhythms could also involve the 5-HT$_{2C}$ receptors. The literature supports a role for 5-hydroxytryptaminergic ascending pathways in the coordination of circadian rhythms to light stimuli. Indeed, the SCN receives a dense 5-hydroxytryptaminergic projection from the midbrain raphe nuclei (van de Kar and Lorens, 1979; Pickard and Rea, 1997) and 5-HT$_{2C}$ receptors have been visualized in it by immunocytochemistry (Moyer and Kennaway, 1999). These observations have prompted the hypothesis that 5-hydroxytryptaminergic innervation of the SCN may serve to modulate the photic response of the SCN circadian oscillator (Pickard and Rea, 1997; Morin, 1999). *In vitro* studies on SCN firing rate rhythms in rats and *in vivo* experimentation on wheel running behaviour in hamsters have confirmed this hypothesis. The involvement of 5-HT$_{2C}$ receptors comes from experiments using the 5-HT$_{2A/2C}$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), which provokes a transient inhibition of melatonin production in rats (Kennaway and Moyer, 1998; Kennaway *et al.*, 2001). The effect of DOI is counteracted by SB 242,084, therefore suggesting an effect mediated by 5-HT$_{2C}$ receptors. Moreover, 5-HT$_{2C}$ receptors agonists can enhance N-acetyl transferase activity and melatonin synthesis in the rat pineal gland, and these effects are prevented by pretreatment with 5-HT$_{2C}$ receptors antagonists (Steardo *et al.*, 2000). These findings support a role for 5-HT$_{2C}$ receptors in the modulation of circadian messages at the level of the pineal gland.

. . . to molecular events

The discovery of the molecular clock machinery provides novel mechanistic insights into how rhythm dysfunction could have drastic behavioural consequences. Endogenous rhythm is generated within SCN cells through the positive and negative feedback actions of several circadian locomotor

cycles kaput (*CLOCK*) gene transcription factors. The circadian clock is based on a series of interconnected transcriptional positive–negative feedback loops that are regulated over the course of 24 h in the absence of environmental input and form the basis for the establishment and maintenance of circadian rhythms in individual cells. In mammals, the *CLOCK* and brain and muscle Arnt-like protein-1 act as major transcriptional activators by forming a heterodimer that promotes transcription of the Period genes (*Per1*, *Per1* and *Per3*), the cryptochrome genes (*Cry1* and *Cry2*), as well as many other genes by binding to E-box elements in their promoters (Turek, 2007).

There is a link between *CLOCK* gene functions and mood regulation (Turek, 2007; Bunney and Potkin, 2008; Mendlewicz, 2009). Multiple mood states – and other circadian perturbations – develop with mutation in a single *CLOCK* gene: mice with a mutation in the *CLOCK* gene (*CLOCK*Δ19) display a complete behavioural profile that is very similar to bipolar patients in the manic state (Roybal *et al.*, 2007). Indeed, chronic treatment with lithium restores the majority of their abnormal behaviours to wild-type levels. *CLOCK* knockdown circumscribed to the VTA is sufficient to lead to less anxiety in the elevated plus maze test and results in a substantial increase in depression-like behaviour, creating, according to the authors, an overall mixed manic state (Mukherjee *et al.*, 2010). Of course, while anxiety-related behaviours can mostly be controlled through *CLOCK* expression in the VTA, it is likely that other brain regions contribute to the regulation of depression-like behaviour.

Some of the effects of agomelatine, notably those on the circadian clock, are presumably linked to changes in the gene transcriptional/translational feedback loop. Rhythmic expression of *CLOCK* genes is seen not only in the SCN, but also outside these nuclei, in brain regions such as the dentate gyrus of the hippocampus, the nucleus accumbens and the frontal cortex (Varcoe *et al.*, 2009). In particular, the chronic administration of agomelatine was found to up-regulate *Rev-erbα, Rev-erbβ* and *Prokineticin2* expression in the SCN, and to modify *Per1* and *Rev-erbα* expression in the frontal cortex in a time-dependent manner; however, no changes in the expression of *Per1*, *Per1*, *CLOCK*, *Bmal1* and *Npas2* genes were noted in the dentate gyrus (Varcoe *et al.*, 2009).

When examining the relationship between PTSD-like behavioural response patterns and *CLOCK* genes in rats, a significant association was found between the degree of behavioural disruption resulting from predator scent stress exposure, and long-term changes in *Per1* and *Per2* gene expression in hippocampal subregions, frontal cortex and SCN areas (Koresh *et al.*, 2012). Changes in the rhythmic expression of *Per1* and *Per2* could affect the functional integrity of cells, and desynchronization among cell populations within the brain may contribute to psychopathology (Wang *et al.*, 2009). When animals are treated with agomelatine (50 mg·kg^{-1} for 3 days) immediately after stress exposure, a reduction in the percentage of individuals with the most severe PTSD-like behavioural stress response is observed up to 7 days following stress. This effect of agomelatine is accompanied by changes in the expressions of *Per1* and *Per1* genes that differ between brain regions: agomelatine induces a decrease in *Per1* and *Per1* mRNA in the hippocampal sub-areas and in the SCN, and an increase in *Per1* mRNA levels in the frontal cortex (Koresh *et al.*, 2012).

Cellular and synaptic effects of agomelatine

Effects linked to neurogenesis

There is compelling neurobiological evidence to suggest that mood disorders are characterized by neuron dendrite shrinkage, glial cell loss, and/or impairments in neuroplasticity and cellular resilience (Tang *et al.*, 2012). The recent findings described later indicate that agomelatine exerts some effects on neurogenesis that may participate in its psychotropic actions. The pro-cognitive effects of agomelatine may also be mediated through the up-regulation of neuroplastic mechanisms as the magnitude of the hippocampal shrinkage reported in certain experimental conditions may partly underlie some of the cognitive deficits that accompany major depression.

Antidepressant treatments increase neural plasticity and adult neurogenesis, especially in the hippocampus (Banasr and Duman, 2007). Chronic agomelatine, like other antidepressants, increases cell proliferation, neurogenesis and cell survival in the hippocampus of rats (Table 3). Chronic (3 weeks), but not acute (4 h) or subchronic (1 week) administration of agomelatine (40 mg·kg^{-1}, i.p.) increases cell proliferation and neurogenesis in the ventral dentate gyrus, a region implicated in emotion processing (Banasr *et al.*, 2006; Soumier *et al.*, 2009). Moreover, agomelatine increases cell maturation and survival in the whole hippocampus (Soumier *et al.*, 2009). Some of these effects need a synergistic action at both MT_1/MT_2 and 5-HT_{2C} receptor subtypes. Thus, 5-HT_{2C} receptor antagonists such as SB243, 213 and S 32006, but not melatonin, can mimic the effects of agomelatine on cell proliferation, while the promoting effect of agomelatine on cell survival is not reproduced by the 5-HT_{2C} receptor antagonists or melatonin alone, but is antagonized by the melatonin antagonist S 22153 (Soumier *et al.*, 2009).

In a depression-like mouse model in which the response of the HPA axis is blunted by corticosterone treatment *via* drinking water (David *et al.*, 2009), a 4 week treatment with agomelatine (10 or 40 mg·kg^{-1} i.p.) can reverse the decrease in hippocampal cell proliferation and increase the maturation of newborn neurons. These neuroplastic effects of agomelatine occur together with an improved depressive/anxiety-like phenotype of the mice, and a normalization of their disturbed circadian rhythms (Rainer *et al.*, 2012).

Agomelatine also reverses the neuroplastic changes and helpless behaviour associated with HPA axis alterations in the transgenic GR-i mouse model (Paizanis *et al.*, 2010). In particular, a 21 day treatment of these mice with agomelatine increases the survival of newly formed cells in the ventral part of the hippocampus without changing their phenotypic differentiation into neurons.

Accordingly, under stress-induced conditions, agomelatine administered for several weeks extends the increased survival of newly formed neurons to the entire dentate gyrus. A 3–6 week treatment with agomelatine (40 mg·kg^{-1}, i.p.) reverses the reduction of neurogenesis induced in the ventral

Table 3

Effects of agomelatine on neuroplasticity. Potential synergy between melatonergic agonist and 5-HT_{2C} antagonist properties

Model	Agomelatine	Melatonin	5-HT_{2C} antagonists	Melatonin antagonist#
Cell maturation survival(1)	+	–	–	Antagonism
Growth factors and Cell signalling				
BDNF in the prefrontal cortex (acute)(2)	+	–	–	Antagonism
BDNF in the hippocampus (chronic)(1)	+	–	–	Not tested
pAKT-pGSK3β in the hippocampus (chronic)(1)	+	+(3)	–	Not tested
Early genes				
Arc in the frontal cortex (acute)(4)	+	–	–	Antagonism
Glutamate release				
Prefrontal/Frontal cortex(5)	+	–	±	Not tested
	Full spectrum of antidepressant activity	Melatonergic effect not sufficient	5-HT_{2C} antagonism not sufficient	

+effect; –no effect; #S22153.
(1) (Soumier *et al.*, 2009); (2) (Molteni *et al.*, 2010); (3) (Lee *et al.*, 2006; Tajes *et al.*, 2009); (4) (Racagni *et al.*, 2011); (5) (Tardito *et al.*, 2010).

hippocampus of rats after prenatal restraint stress (Morley-Fletcher *et al.*, 2011). In a chronic foot shock stress paradigm, c-Fos expression is reduced in the dentate gyrus, and chronic agomelatine treatment reverses this effect and normalizes neuronal activity to basal levels. Chronic agomelatine also enhances hippocampal cell proliferation in rats concurrently exposed to chronic stress and promotes survival of the newly born cells selectively in the ventral hippocampus (Dagyte *et al.*, 2010). Finally, agomelatine treatment reverses the stress-induced decrease in doublecortin (DCX) expression. Taken together, these data indicate that the benefit of agomelatine's action in situations of stress, and its antidepressant activity, may depend on the ability to reverse stress-affected neuronal activity and promote hippocampal neurogenesis (Dagyte *et al.*, 2010). The effects of agomelatine on hippocampal neurogenesis have also been investigated in the situation of anhedonia in the chronic mild stress paradigm. While chronic mild stress significantly decreases the newborn cell survival and DCX expression in the dentate gyrus, agomelatine normalizes stress-affected cell survival and partly restores DCX expression to non-stress levels (Dagyte *et al.*, 2011). Finally, the suppression of neurogenesis by hippocampus irradiation eliminated the antidepressant effects of agomelatine in the novelty-suppressed feeding paradigm (David *et al.*, 2008).

Depression has been associated with reduced expression of brain-derived neurotrophic factor (BDNF) in the hippocampus. Associations between reduced hippocampal neurogenesis and depressive-like behaviour are mainly based on indirect findings such as responses to antidepressant drugs and alterations in BDNF levels and neurogenesis in depressed patients or animal models of depressive behaviour (Shirayama *et al.*, 2002; Hoshaw *et al.*, 2005). Substantial support for the neurotrophic hypothesis of depression is brought by the finding that BDNF knocking down circumscribed to the dorsal dentate gyrus can reduce neuronal differentiation (but not proliferation) (Taliaz *et al.*, 2010). Several behavioural impairments are also noted after the knocking down of neurotrophin expression, specifically in the ventral subiculum, notably an anhedonic-like behaviour. Elevation in hippocampal BDNF expression apparently plays key roles in the behavioural effects of various antidepressant treatments (Taliaz *et al.*, 2011). As for many other antidepressants that have demonstrated a positive impact on neurogenesis, a neurotrophic factor is probably involved in the neurogenetic effects of agomelatine. Chronic agomelatine administration increases whole hippocampal mRNA (Calabrese *et al.*, 2011) and protein contents of BDNF (Soumier *et al.*, 2009; Ladurelle *et al.*, 2012). A similar effect has been seen in the transgenic GR-i mouse model (Paizanis *et al.*, 2010) as, together with helpless behaviour associated with HPA axis alterations, chronic agomelatine reverses the down-regulated expression of BDNF mRNA in these animals. Recent experimental data in the GR-i mouse model revealed the antagonism of certain behavioural and neuroplastic effects of agomelatine by inhibitors of trkB (tropomyosin-related kinase) receptors (L. Lanfumey, pers. comm.).

Acute administration of agomelatine increases BDNF mRNA levels in the prefrontal cortex of rats; this effect relies on the synergistic activity of the antidepressant at MT_1/MT_2 and 5-HT_{2C} receptors, as it cannot be replicated by either melatonin or a 5-HT_{2C} antagonist, when given separately, but is antagonized by the melatonin antagonist S 22153 (Molteni *et al.*, 2010) (Table 3).

The list of molecular players involved in depression's phenotypes has expanded to include genes from diverse aspects of cellular physiology, especially transcription factors, p11, annexins, proteins of the S-100 family, and many others. Of course, a challenge certainly is to determine whether or not the targets themselves may be therapeutically relevant for human depression. Part of the response relies on the demonstration that these newly identified factors are

involved in the mechanistic foundation of existing therapeutics. For example, the activity-regulated cytoskeleton-associated protein (Arc) is another mediator of neuroplastic response relevant to the chronic action of various antidepressants (Pei *et al.*, 2003). The expression of this immediate early gene is known to follow significant time-of-day changes in specific brain regions such as the SCN, the frontal cortex and the dentate gyrus (Varcoe *et al.*, 2009). Arc can be involved in the action of agomelatine as chronic administration of the antidepressant up-regulates *Arc* mRNA expression in whole hippocampus (Calabrese *et al.*, 2011).

The ERK-cAMP-responsive-element-binding protein (CREB) signal system is involved in the molecular pathways of depression as well as in the mechanism of action of some antidepressants (Almeida *et al.*, 2006; Gourley *et al.*, 2008; Qi *et al.*, 2008; Musazzi *et al.*, 2010a; Galeotti and Ghelardini, 2012). Accordingly, in the model of prenatal stress, adult rats have reduced hippocampal levels of phosphorylated CREB (p-CREB), as expected of a depressive phenotype. In this model, p-CREB signalling returns to control level after the chronic administration of agomelatine (40 mg·kg^{-1}), indicating that this pathway is involved in the action of the compound (Morley-Fletcher *et al.*, 2011).

The antidepressant and procognitive actions of agomelatine are accompanied by modulation of the kinase microtubule-associated protein 2, as well as cytoskeletal microtubule dynamics and synaptic markers such as synaptophysin, postsynaptic density-95 (PSD-95), spinophilin and BDNF in the hippocampus, amygdala and PFC in a region-specific pattern (Ladurelle *et al.*, 2012). In particular, the expression of the dendrite-specific MAP-2 and its phosphorylation at Ser^{136} were both increased by chronic agomelatine in the hippocampus and at a much higher level in the amygdala. These findings, which may be related to regulation of microtubule dynamics and/or dendritic remodelling, as also seen with fluoxetine (Bessa *et al.*, 2009), are in agreement with the findings that (sub)chronic administration of different classes of antidepressants (imipramine, desipramine, fluvoxamine) can affect MAP-2 expression and/or phosphorylation in rat brain regions (Perez *et al.*, 1995; Miyamoto *et al.*, 1997; Iwata *et al.*, 2006). The NMDA receptor antagonist ketamine, endowed with a rapid antidepressant action (Machado-Vieira *et al.*, 2009), similarly increased PSD-95 and synapsin I expressions, resulting in synaptic strengthening (Li *et al.*, 2010; Li and Jope, 2010).

Chronic agomelatine administration to rats potentiates the phosphorylation of the β isoform of glycogen synthase kinase-3 (GSK3β) in the total hippocampus (Soumier *et al.*, 2009) (Table 3). GSK3β exerts profound influences on cellular architecture and plasticity. GSK3β can be inhibited by phosphorylation through several kinases, and an impairment of this inhibitory control can result in abnormally high GSK3β activity, a condition that can have detrimental effects on neuronal plasticity, structure and survival (Jope and Roh, 2006). GSK-3β can be phosphorylated by ERK1/2 and agomelatine does activate these kinases that are implicated in cell survival mechanisms (Soumier *et al.*, 2009). GSK3β has received much attention because the mood stabilizer lithium is a direct inhibitor of this enzyme, a finding that raised the possibility that GSK3β may not be adequately controlled in mood disorders (Jope and Roh, 2006; Li and Jope, 2010). GSK3β could represent another potential connection between circadian rhythms and treatment of mood disorders as it is one of several kinases involved in the regulation of circadian cycles through the phosphorylation of *Per* and other circadian gene products (Takahashi, 2004). Clearly, it is important to clarify further the possible link between the action of agomelatine and the expression of this kinase because a dys-regulated GSK3β pathway may also have multiple effects that could impair neural plasticity, such as modulation of neuronal architecture, neurogenesis, gene expression and the resilience of neurons to stress, all of which have also been associated with the mechanism of action of the compound. BDNF also plays a role in the modulation of circadian rhythms and fluctuations of the expression of this neurotrophic factor and its receptor occur over the course of a circadian cycle (Cirulli and Alleva, 2009). Besides, it has been hypothesized that GSK3β may be a central regulator of the endogenous circadian clock. The link between the chronobiotic actions of agomelatine and the mediators of neuroplastic responses and signalling pathways mentioned earlier therefore supports the hypothesized mode of action of the compound (Racagni *et al.*, 2011).

Involvement of the glutamate pathway

The impact of agomelatine on glutamate signalling has been examined as this excitatory amino acid neurotransmitter and its receptors play a role in depression and antidepressant activity (Mitchell and Baker, 2010). Drugs with glutamate-based mechanisms are endowed with accelerated onset of activity. Currently, the non-competitive NMDA receptor antagonist ketamine appears an attractive therapeutic agent for treatment-resistant patients with depressive disorder (Machado-Vieira *et al.*, 2009). However, the cognitive and dissociative side effects of ketamine limit its widespread application. The exact implication of glutamate in depression and antidepressant treatment is far from clear because of the many interactions with other neurotransmitter systems, for example through kinase phosphorylation and BDNF synthesis in the case of ketamine (Autry *et al.*, 2011). A number of other drugs [e.g. selective GluN2B receptor (NR2B) antagonists, positive modulators of AMPA receptors, and metabotropic glutamate (mGlu) receptor agonists/antagonists for nomenclature see Alexander *et al.*, 2013a] also exhibit antidepressant-like effects in animal models of depression (Hashimoto, 2011), but no clinical studies demonstrating the effectiveness of these drugs are available.

Through a synergistic interaction between its MT_1/MT_2 and $5\text{-}HT_{2C}$ receptor components, agomelatine modulates glutamate signalling, engaging time-dependent modifications of receptors and transporters in circumscribed regions involved in the regulation of mood, circadian rhythms and cognition (Racagni *et al.*, 2011). Thus, the acute administration of agomelatine (40 mg·kg^{-1} i.p.) abolishes the restraint stress-induced increase in extracellular glutamate efflux in limbic structures such as the basolateral and central nuclei of the amygdala and the hippocampus (Reagan *et al.*, 2012). As previously found with other antidepressants (Bonanno *et al.*, 2005), chronic administration of agomelatine (40 mg·kg^{-1}, i.p., for 21 days) significantly reduced endogenous release of glutamate from hippocampal synaptosomes and decreased

the accumulation of SNARE (soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor) complex, a key molecular effector of vesicle docking, priming and fusion at presynaptic membranes (Milanese *et al.*, 2013). Long-term administration of antidepressants elicits adaptive changes in the functional status of mGlu receptors (Paul and Skolnick, 2003; Palucha and Pilc, 2007). This is also the case of chronic agomelatine, which normalizes glutamate release and the expression of $mGlu_{2/3}$ and $mGlu_5$ receptor mRNAs in the hippocampus of rats subjected to prenatal restraint stress (Morley-Fletcher *et al.*, 2011; Marrocco *et al.*, 2014). Further, chronic agomelatine treatment modulates the expression of mGlu receptor mRNA in a time- and region-dependent manner (Varcoe *et al.*, 2009). For example, agomelatine increases the evening expression of $mGlu_1$, $mGlu_4$, $mGlu_6$ and $mGlu_8$ receptors in the frontal cortex, while some transcripts can be over-expressed in the morning ($mGlu_7$) and at night ($mGlu_8$). Other time-dependent changes are induced by agomelatine, for example in the SCN (where $mGlu_7$ receptor levels increased at night and decreased during subjective day). Those changes in the timing of glutamate receptors could be conveyed by rhythmicity of certain *CLOCK* gene's transcription factors (Varcoe *et al.*, 2009).

The mGlu receptor subtypes cited earlier are negatively coupled to AC, are expressed by both neuronal and glial cells, and are predominantly localized to axon terminals. Thus, any increased expression of these receptors may be associated with the fact that chronic agomelatine also reduces glutamate release in the prefrontal/frontal cortex of rats exposed to acute footshock stress (Musazzi *et al.*, 2010b; Tardito *et al.*, 2010). The latter effect of agomelatine on glutamate signalling is again coupled to its synergistic action on melatonergic and 5-hydroxytryptaminergic pathways since it is only partly replicated by 5-HT_{2C} receptor blockade with S 32006, and cannot be obtained after melatonin treatment (Tardito *et al.*, 2010) (Table 3).

The vesicular glutamate transporters (vGLUTs; Alexander *et al.*, 2013b) are involved in the loading of glutamate into synaptic vesicles. A reduced expression of these transporters has been linked to depressive behaviour in both clinical and preclinical studies. Specifically, there is evidence that the expression of the vGLUT type 1 (vGLUT1) is reduced in an animal model of depression (Zink *et al.*, 2010), and knocking down vGLUT1 expression provokes depressive-like behaviours in mice (Tordera *et al.*, 2007). In addition, reduced expression of vGLUT1 and vGLUT2 has been observed in *post mortem* brains of depressed subjects (Uezato *et al.*, 2009). The expression of vGLUT1 mRNA is up-regulated in the frontal cortex following chronic agomelatine treatment (Varcoe *et al.*, 2009). Similar effects have been reported with other antidepressant classes in rodents (Moutsimilli *et al.*, 2005; Tordera *et al.*, 2005). In addition, chronic agomelatine treatment modified the pattern of expression of vGLUT2 mRNA in the SCN and frontal cortex, with an increased expression during the night, and a decrease during the subjective day (Varcoe *et al.*, 2009).

Finally, pro-cognitive effects of agomelatine may be mediated through glutamatergic processes involved in the induction of long-term potentiation and in memory formation, possibly by normalizing stress-induced alterations in the glutamatergic system (Musazzi *et al.*, 2010b; Tardito *et al.*, 2010).

Effects on inflammatory markers

Specific components of the immune/inflammatory system play a crucial role in the antidepressant response and thereby in depression aetiopathology. Chronic treatment with agomelatine significantly reduced the LPS-induced up-regulation of the pro-inflammatory cytokines IL-1β and IL-6 in the rat brain as well as at peripheral level (Molteni *et al.*, 2013). Agomelatine also altered the expression of enzymes related to the kynurenine pathway thought to represent important mediators in inflammation-related depression. Thus, agomelatine appears to interfere with molecular systems involved with inflammatory responses.

Conclusion

Agomelatine exerts psychotropic effects upon mood and anxious states. The psychotropic actions of agomelatine in a range of experimental models are based on a synergistic interplay of its melatonergic agonist and 5-HT_{2C} receptor neutral antagonist actions (Racagni *et al.*, 2011). If confirmed *in vivo*, the demonstration of a 'crosstalk' between these different receptors would represent a major step forward in the understanding of this synergistic action (Kamal *et al.*, 2010). Our knowledge concerning the mechanisms of action and functional properties of agomelatine is being progressively enhanced. Agomelatine affects a number of neurobiological events including transcriptional-translational molecular circadian clock, neurogenesis, synaptic remodelling and glutamate signalling. It will be important to further characterize these properties, and to explore other possible mechanisms of action in order to deepen our understanding of the neurobiological substrates underpinning the distinctive functional and therapeutic profile of agomelatine.

Conflict of interest

All authors are full-time employees of Servier and have no other interests to declare.

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