European Journal of Pharmacology 588 (2008) 58–63

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Agomelatine, a melatonin receptor agonist with 5-HT_{2C} receptor antagonist properties, protects the developing murine white matter against excitotoxicity

Pierre Gressens [a,b,c,d,*], Leslie Schwendimann [a,b,d], Isabelle Husson [a,b,c,d], Gergely Sarkozy [e], Elizabeth Mocaer [f], Joseph Vamecq [g], Michael Spedding [f]

[a] Inserm, U676, Paris, F-75019, France
[b] Université Paris 7, Faculté de Médecine Denis Diderot, IFR02 and IFR25, Paris, France
[c] AP HP, Hôpital Robert Debré, Service de Neurologie Pédiatrique, Paris, France
[d] PremUP, Paris, France
[e] Department of Neonatology, Medical University Innsbruck, Innsbruck, Austria
[f] Institut de Recherches Servier (IdRS), 11 Rue des Moulineaux, 92150, Suresnes, France
[g] Inserm UNIV 045131, Neuropédiatrie, Hôpital Salengro, CHRU, Lille, France

ARTICLE INFO

Article history:
Received 20 June 2007
Received in revised form 17 March 2008
Accepted 3 April 2008
Available online 8 April 2008

Keywords:
Cerebral palsy
Periventricular leukomalacia
N-methyl-d-aspartate
Ibotenate
Agomelatine

ABSTRACT

Periventricular leukomalacia is a major cause of cerebral palsy. Perinatal white matter lesions associated with cerebral palsy appears to involve glutamate excitotoxicity. When injected intracerebrally into newborn mice, the glutamatergic analog, ibotenate, induces white matter cysts mimicking human periventricular leukomalacia. Intraperitoneal injection of melatonin was previously shown to be neuroprotective in this mouse model. The goal of the present study was to compare in this model the protective effects of agomelatine (S 20098), a melatonin derivative, with melatonin. Mice that received intraperitoneal S 20098 or melatonin had significant reductions in size of ibotenate-induced white matter cysts when compared with controls. Although agomelatine and melatonin did not prevent the initial appearance of white matter lesions, they did promote secondary lesion repair. Interestingly, while melatonin effects were only observed when given within the first two hours following the excitotoxic insult, agomelatine was still significantly neuroprotective when administered eight hours after the insult. The protective effects of agomelatine and melatonin were counter-acted by co-administration of luzindole or S 20928, two melatonin receptor antagonists. Agomelatine, acting through melatonin receptors, could represent a promising new drug for treating human periventricular leukomalacia and have beneficial effects on neuroplasticity.

1. Introduction

Full-term human neonates with perinatal encephalopathy generally develop gray matter damage that most frequently affects the neocortex, the basal ganglia, and the hippocampus (Volpe, 2001). Periventricular leukomalacia, which consists of focal, often cystic, necrosis of periventricular white matter, is most frequently observed in human pre-term neonates (Volpe, 2001). Periventricular leukomalacia, due to the increased rate of multiple pregnancies and the increased survival of very or extremely pre-term infants (Hagberg et al., 1996), is a major cause of cerebral palsy. Despite major improvements in neonatal care, there is still no specific treatment for periventricular leukomalacia.

In recent years, the causes of brain injury in human neonates have been considered to be multifactorial (Dammann and Leviton, 1997; Grether et al., 1996). Many pre-conceptional, prenatal, and perinatal factors are thought to cause injury to the developing brain, such as hypoxic-ischemic insults, endocrine imbalances, genetic factors, growth factor deficiency, abnormal competition for growth factors, maternal infection yielding excess cytokines, and exposure to other pro-inflammatory agents. Several of these risk factors may share the same molecular pathways, such as excess release of excitatory amino acids and excess reactive oxygen species production (Haynes et al., 2003; Plaisant et al., 2003; Loeliger et al., 2003). Accordingly, several laboratories have shown that intracerebral injection of glutamate analogs in newborn rodents produces striatal and cortical plate damage mimicking those observed in full-term human infants, as well as cystic periventricular white matter lesions that mimic those observed in human pre-term neonates (Acarin et al., 1999; Barks and Silverstein, 1992; Dommergues et al., 2000, 2003; Follett et al., 2000; Gressens et al., 1997; Husson et al., 2002, 2005; Marret et al., 1995; McDonald et al., 1998; Tahraoui et al., 2001).

* Corresponding author. Hopital Robert Debre, INSERM U 676, 48 Blvd Serurier, 75019 Paris, France. Tel.: +33 1 40 03 19 76; fax: +33 1 40 03 19 95.
E-mail address: pierre.gressens@inserm.fr (P. Gressens).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.04.016

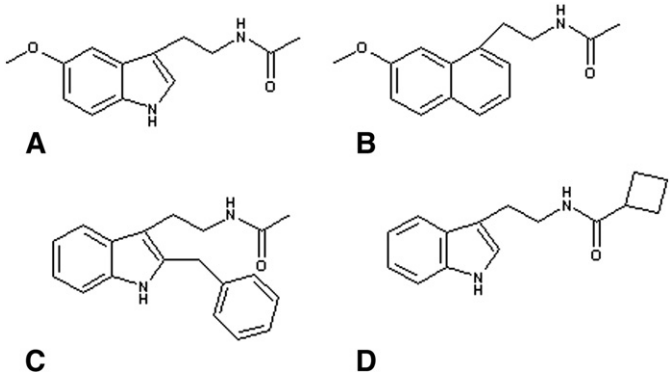

Fig. 1. Chemical structure of melatonin (A), agomelatine (B), and the melatonin MT receptor antagonists luzindole (C) and S 20289 (D).

Using such a model of neonatal excitotoxic brain lesions in newborn mice, we previously showed that intraperitoneal melatonin induced an 82% reduction in size of NMDA receptor-mediated white matter cysts when compared with controls (Husson et al., 2002). Although melatonin did not prevent the initial appearance of white matter lesions, it did promote secondary lesion repair. Axonal markers supported the hypothesis that melatonin induced axonal regrowth or sprouting. The protective effects of melatonin were suppressed by co-administration of luzindole, a mixed melatonin MT_1/MT_2 receptor antagonist. Therefore, melatonin and derivatives acting through activation of melatonin receptors could represent new avenues for treating human periventricular leukomalacia.

Agomelatine (S 20098; *N*[2-(7-methoxy-1-naphthyl) ethyl] acetamide) is a new molecule active in preclinical models predictive of antidepressant properties (Bertaina-Anglade et al., 2002; Papp et al., 2003) and with demonstrated clinical efficacy in major depressive disorders (Loo et al., 2002, 2003; Olié and Kasper, 2007). Studies of the interactions of agomelatine with a wide range (>80) of receptors and enzymes showed that this compound has negligible affinity ($IC_{50}>10^{-5}$ M) for all these potential targets except melatonin MT_1 and MT_2 ($K_i=6.2\times10^{-11}$ and 2.7×10^{-10} M, respectively) (Conway et al., 2000) and serotonin 5-HT_{2C} ($IC_{50}=2.7\times10^{-7}$ M) receptors (Millan et al., 2003). Especially also at glutamate, kainate and NMDA-sites the IC_{50}s for agomelatine were >10–5 M.

The goal of the present study was to compare the neuroprotective effects of agomelatine and melatonin against NMDA receptor-mediated white matter lesions in newborn mice.

2. Materials and methods

Swiss pups (Elevage Janvier, Le Genest-St-Isle, France) were used in all experiments. Experimental protocols were approved by the institutional review committee, meet the INSERM guidelines, and were carried out in accordance with the Guide for the Care and use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

2.1. Excitotoxic insult and lesion size determination

Lesions were induced by injecting 10 µg ibotenate (Tocris, Bristol, UK) into developing mouse brains. Ibotenate activates *N*-methyl-D-aspartate (NMDA) and metabotropic receptors but not alpha-3-amino-hydroxy-5-methl-4-isoxazole (AMPA) and kainate receptors. As previously described (Dommergues et al., 2000; Gressens et al., 1997; Husson et al., 2002; Laudenbach et al., 2001; Marret et al., 1995; Tahraoui et al., 2001), anesthetized mouse pups were injected intracerebrally (i.c.) at postnatal day five (P5) between 9:00 and 10:00 AM. The 25-gauge needle was inserted 2 mm under the scalp skin in the fronto-parietal area of the right hemisphere, 2 mm from the midline in the lateral-medial plane and 3 mm (in the rostro-caudal plane) from the junction between the sagittal and lambdoid sutures. Histopathology confirmed that the tip of the needle always reached the periventricular white matter. Two 1 µl boluses of ibotenate were injected at a 20-second interval. The needle was left in place for an additional 20 s.

Mouse pups were sacrificed by decapitation eight, 24, 72, and 120 h following the excitotoxic challenge. Brains were immediately fixed in 4% formalin for seven days. Following paraffin embedding, we cut 15 µm thick coronal sections. Every third section was stained with cresyl-violet. In theory, neocortical and white matter lesions can be defined by the maximal length of three orthogonal axes: the lateral-medial axis (in a coronal plane), the radial axis (also in a coronal plane, from the pial surface to the lateral ventricle) and the fronto-occipital axis (in a sagittal plane). Due to the difficulty of accurately evaluating the degree of damage to neurons in neocortical layers in the epicenter of the lesion focus, the radial axis did not appear as an objective measure of the lesion size. In previous studies (Gressens et al., 1997; Husson et al., 2002; Marret et al., 1995), we had shown an excellent correlation between the maximal size of the lateral-medial and fronto-occipital diameters of the excitotoxic lesions. Based on these observations, we serially sectioned the entire

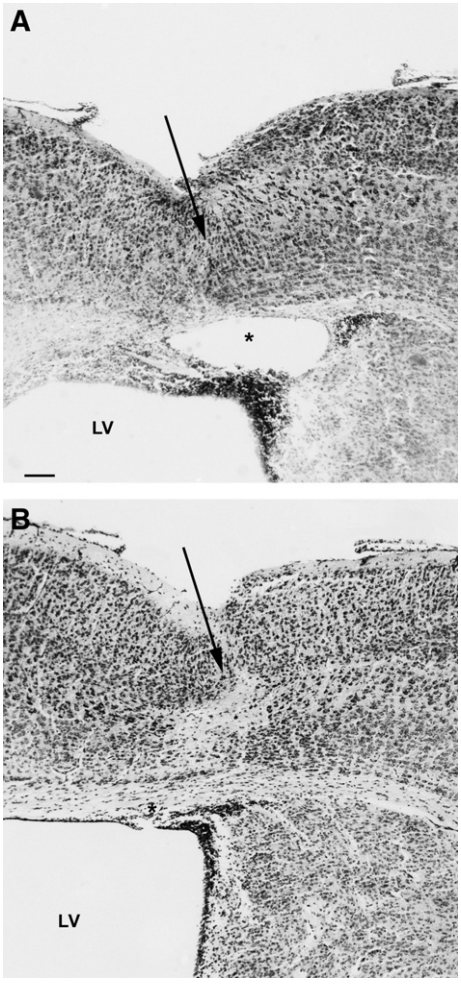

Fig. 2. Agomelatine protects the periventricular white matter against ibotenate-induced lesions. Cresyl violet-stained sections showing brain lesions induced by ibotenate injected (10 µg i.c.) at P5 and studied at the age of P10. (A) Brain from pup co-treated with intracerebral ibotenate and intraperitoneal PBS, showing the typical neuronal loss in layers II–VI (arrow) and the white matter cystic lesion ("c" and arrowheads). (B) Brain from pup co-treated with intracerebral ibotenate and intraperitoneal agomelatine (S 20098). LV, lateral ventricle. Bar: 40 µm.

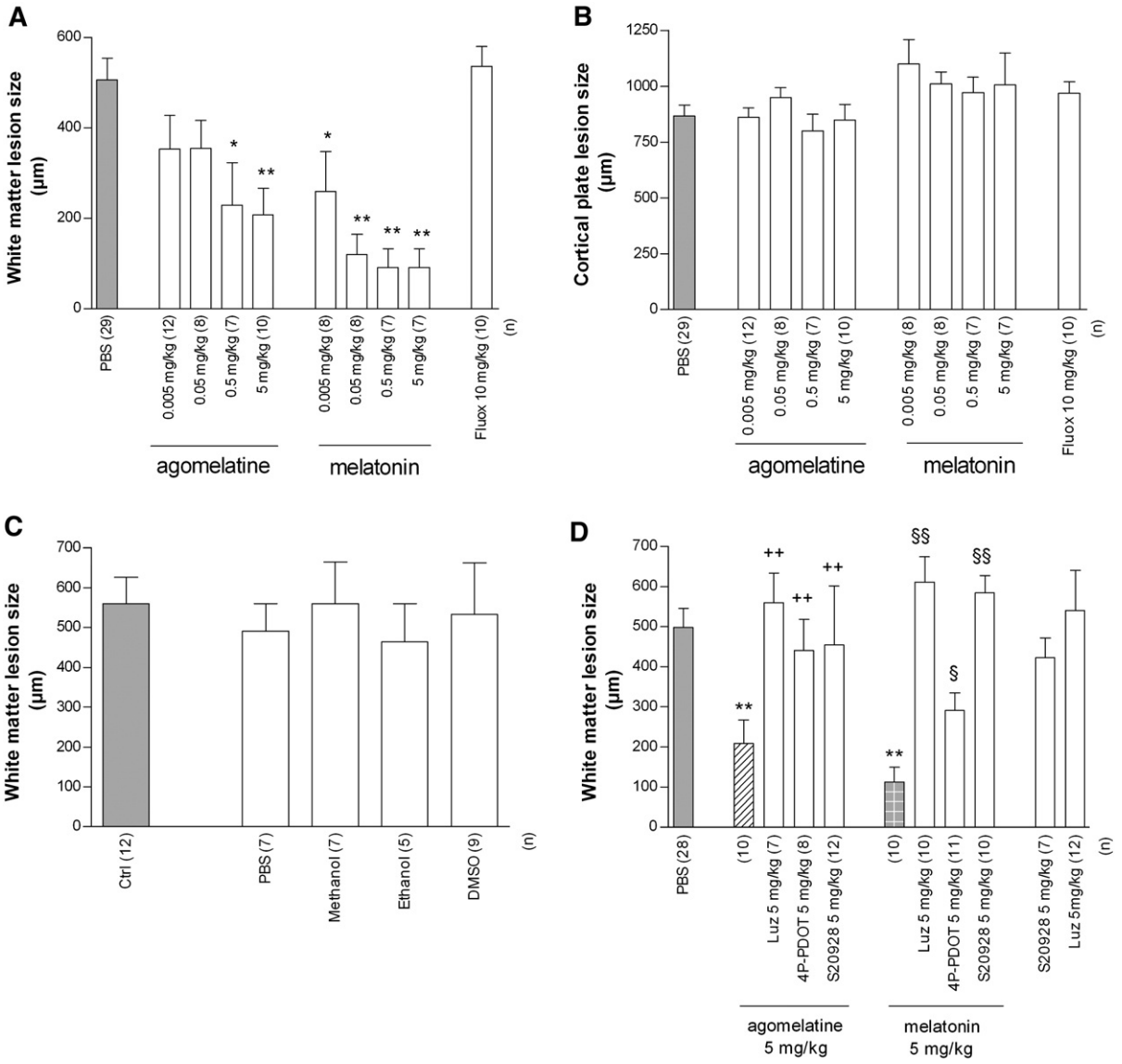

Fig. 3. (A, B) Effects of intraperitoneal agomelatine, melatonin, and fluoxetine (Fluox) administered immediately after 10 µg i.c. ibotenate on excitotoxic white matter (A) and cortical plate (B) lesions. Pups were killed on P10. (C) Effects of intraperitoneal administration of vehicles immediately after 10 µg i.c. ibotenate on excitotoxic white matter lesions. Pups were killed on P10. (D) Effects of intraperitoneal administration of melatonin receptor agonists and antagonists immediately after 10 µg i.c. ibotenate on excitotoxic white matter lesions. Pups were killed on P10. Luz, luzindole. Bars represent mean length of the lesions ± S.E.M. Asterisks indicate difference from grey (*), hatched ($^{+}$) or dotted (§) bars; $^{*,\ \S}$ $P<0.05$, $^{**,\ ++,\ \S\S}$ $P<0.01$ in ANOVA with Dunnet's (A) or Bonferroni's (D) multiple comparison test.

brain in the coronal plane. This permitted an accurate and reproducible determination of the maximal sagittal fronto-occipital diameter (which is equal to the number of sections where the lesion was present multiplied by 15 µm) and was used as an index of the volume of the lesion.

2.2. Experimental groups

Pups from at least two different litters were used in each experimental group, and data were obtained from two or more successive experiments.

Agomelatine (Servier), S 20928 (Servier) and 4-phenyl-2-propionomidotetralin (4P-PDOT; Tocris), were diluted in 95% ethanol, luzindole (Sigma, L'Isle d'Abeau Chesnes, France) was diluted in 40% methanol, melatonin (Sigma) and SB206,553 (Sigma) were diluted in PBS containing 40% dimethylsulfoxide (DMSO; Sigma), and fluoxetine (Sigma) was diluted in PBS.

In a first set of experiments, immediately after i.c. injection with ibotenate, P5 pups received an i.p. injection with one of the following drugs or combination of drugs diluted in a final volume of 5 µl: 0.005 to 5 mg/kg agomelatine; 0.005 to 5 mg/kg melatonin; 10 mg/kg fluoxetine; 5 mg/kg S 20928; 5 mg/kg luzindole; 5 mg/kg agomelatine+5 mg/kg luzindole; 5 mg/kg agomelatine+5 mg/kg S 20928; 5 mg/kg agomelatine+5 mg/kg 4P-PDOT; 5 mg/kg melatonin+5 mg/kg luzindole; 5 mg/kg melatonin+5 mg/kg S 20928; 5 mg/kg melatonin+5 mg/kg 4P-PDOT. Control animals received only i.p. PBS containing 40% DMSO, i.p. PBS containing 40% methanol, i.p. PBS containing 95% ethanol, i.p. PBS alone or no i.p. injection.

In a second set of experiments, immediately after (T0h), two hours (T2h), four hours (T4h) or eight hours (T8h) following i.c. injection with ibotenate, P5 pups received an i.p. injection with one of the following drugs or combination of drugs diluted in a final volume of 5 µl: 5 mg/kg agomelatine; 5 mg/kg melatonin; 5 mg/kg agomelatine+5 mg/kg luzindole; 5 mg/kg melatonin+5 mg/kg luzindole; PBS alone.

In a third set of experiments, concomitantly to (T0 h) or eight hours (T8h) following i.c. injection with ibotenate, P5 pups received an i.p. injection of 5 mg/kg agomelatine or 5 mg/kg melatonin

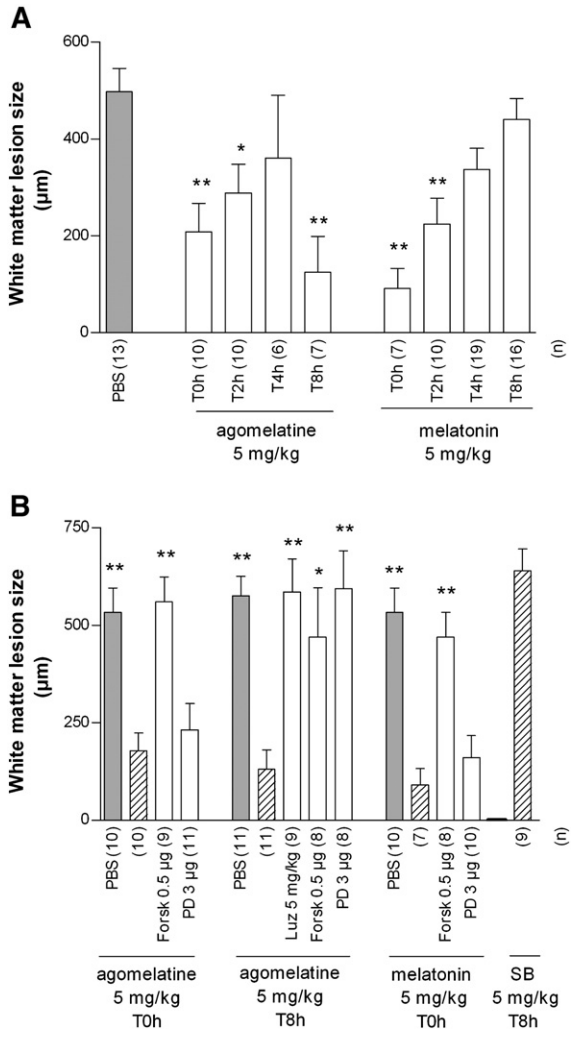

Fig. 4. (A) Effects of delayed (times are indicated on the *X* axis) intraperitoneal administration of agomelatine or melatonin on ibotenate-induced white matter lesions. Pups were killed on P10. (B) Effects of intracerebral forskolin (Forsk), intracerebral PD98059 (PD), intraperitoneal luzindole (Luz) or intraperitoneal SB206,553 (SB) on neuroprotection induced by melatonin or agomelatine intraperitoneally administered immediately (agomelatine T0h and melatonin T0h) or eight hours (agomelatine T8h) after ibotenate. Pups were killed on P10. Bars represent mean length of the lesions ± S.E.M. Asterisks indicate difference from hatched bars; * $P<0.05$, ** $P<0.01$ in ANOVA with Bonferroni's multiple comparison test.

combined with an i.c. injection of 3 µg PD98059 [2-(2'-Amino-3'-methoxy)-flavone; New England Biolabs, Beverly, MA, USA], or 0.5 µg forskolin (Biomol, Plymouth Meeting, PA, USA). In addition some pups received an i.p.; injection with 5 mg/kg SB206,553 eight hours (T8 h) following i.c. injection with ibotenate.

Luzindole, 4P-PDOT and S 20928 block melatonin MT_1 and MT_2 receptors. Luzindole has comparable affinities for both receptors (affinity selectivity ratio $MT_1/MT_2=15.5$).

4P-PDOT is more MT_2 selective (affinity selectivity ratio $MT_1/MT_2=311$). S 20928 is a potent melatonin MT_1 and MT_2 receptor antagonist (Yang et al., 2001). Structures of the melatonin receptor agonists and antagonists are given in Fig. 1. Forskolin is an adenylate cyclase activator. PD98059 is an inhibitor of extracellular signal-regulated kinase 1 (ERK1) and ERK2. SB206,553 is a selective 5-$HT_{2B/2C}$ receptor antagonist (Bromidge et al., 1998).

2.3. Statistical analyses

Most data were analyzed with a one-way ANOVA with Dunnett's or Bonferroni's multiple comparison tests. In the subset of experiments where white matter lesion size was evaluated at different time points after ibotenate injection, results were studied using two-way ANOVA with Treatments and Age (time elapsed after injection) as between-subject factors with comparisons between treatment groups at each age.

3. Results

Overall, mortality was low (<5%) in pups receiving ibotenate injections. All pups injected with ibotenate displayed tonic-clonic convulsions within the first 24 h following the excitotoxic challenge. Co-treatment with melatonin receptor agonists and antagonists or with fluoxetine did not modify mortality significantly (<5% in all experimental groups) nor did it alter the incidence, severity, or phenotype of convulsions.

Pups injected i.c. with ibotenate and i.p. with PBS on P5 developed cortical lesions and periventricular white matter cysts (Fig. 2A). The cortical lesion was typical, with dramatic neuronal loss in all neocortical layers and almost complete disappearance of neuronal cell bodies along the axis of ibotenate injection. I.p. administration of agomelatine or melatonin produced a dose-dependant reduction (up to 59% and 82%, respectively with 5 mg/kg agomelatine or melatonin) of the ibotenate-induced white matter lesion but did not affect the cortical plate lesion (Figs. 2B and 3A–B). I.p. administration of fluoxetine had no significant effect on ibotenate-induced brain lesions (Fig. 2A–B). The i.p. administration of solvents, 95% ethanol for agomelatine and 40% methanol for melatonin, respectively, had no significant effect on the excitotoxic lesions (Fig. 3C). Neuroprotective effects of 5 mg/kg i.p. doses of agomelatine against white matter lesions were completely abolished by co-treatment with 5 mg/kg i.p. doses of luzindole, 4P-PDOT or S 20928 (Fig. 3D). Neuroprotective effects of melatonin were completely abolished by co-treatment with luzindole or S 20928 and partially reversed by 4P-PDOT (Fig. 3D).

The neuroprotective effect of an i.p. injection of agomelatine or melatonin following the excitotoxic challenge, was time-dependent. Protection of the white matter was observed with melatonin within the first two hours (T0h or T2h) after ibotenate administration (Fig. 4A). Similarly, agomelatine-induced neuroprotection was progressively fading out when administration was delayed to four hours

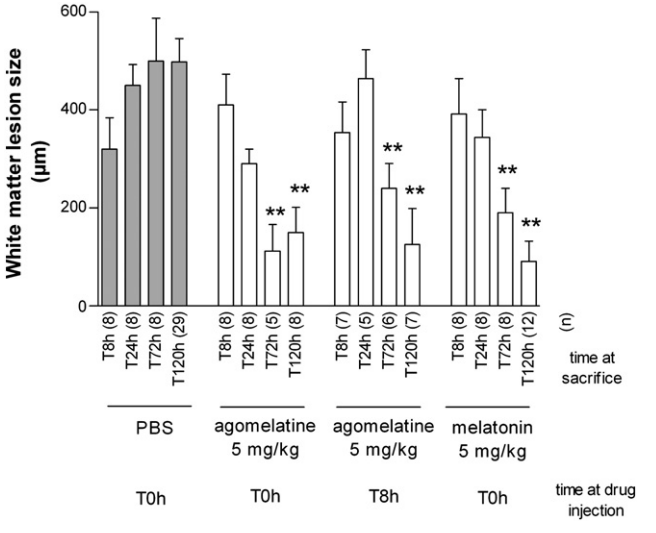

Fig. 5. Effects of intraperitoneal agomelatine or melatonin administered immediately after (agomelatine T0h and melatonin T0h) or eight hours (agomelatine T8h) after ibotenate injection on excitotoxic white matter lesions. Pups were killed at different time, as indicated on the *X* axis (immediately below bars). Bars represent mean length of the lesions ± S.E.M. Group by Age interaction (ANOVA) was significant ($P<0.0001$); asterisks indicate difference from ibotenate-treated animals in contrasts (** $P<0.01$).

(T4h) (Fig. 4A). However, when agomelatine was administered eight hours (T8h) after ibotenate, again a significant neuroprotection of the white matter was observed (Fig. 4A). Co-administration of luzindole suppressed the neuroprotective effects of agomelatine given at T8h (Fig. 4B). In line with a previous report (Husson et al., 2002), co-administration of forskolin prevented the neuroprotective effects of melatonin given at T0h while co-administration of PD98059 had no significant effect (Fig. 4B). Similarly, co-administration of forskolin prevented neuroprotective effects of agomelatine given at T0h while co-administration of PD98059 had again no significant effect (Fig. 4B). In contrast, co-administration of forskolin or PD98059 blocked neuroprotective effects of agomelatine administered at T8h (Fig. 4B). In addition, SB206,533 had no detectable effect on ibotenate-induced lesions showing that 5-HT_{2C} antagonism was not the mechanism of the neuroprotective effects of agomelatine under these experimental conditions.

In animals injected with ibotenate alone, the white matter lesion increased in size during the first 24 h and thereafter remained stable (Fig. 5). Co-treatment with ibotenate and agomelatine or melatonin, or treatment with ibotenate and delayed agomelatine (injected eight hours after the insult) did not modify the lesion size examined after the initial 24 h, but caused a dramatic regression in the size of the white matter lesion over the next four days (Fig. 5).

4. Discussion

The most salient feature of the present study is the demonstration that agomelatine potently protects the developing brain from ibotenate insults. Although agomelatine is slightly less potent than melatonin, the window of opportunity for treatment is much broader than for melatonin.

Interestingly, the agomelatine- and melatonin-induced neuroprotection against an excitotoxic challenge mediated by NMDA receptors was specific for the white matter. It did not affect cortical plate lesions. These findings strongly suggest that white matter lesions are not secondary to cortical plate lesions.

Several lines of evidence suggest that the neuroprotective effects of agomelatine and melatonin are largely mediated by specific melatonin receptors, and not anti-oxidant properties: i) luzindole, 4P-PDOT or S 20928, the melatonin receptor antagonists partially or completely reversed agomelatine and melatonin-induced neuroprotection; ii) forskolin abolished agomelatine and melatonin-induced neuroprotection; iii) the neuroprotective doses of agomelatine and melatonin were lower than those generally displayed a significant anti-oxidant effect; iv) we previously showed that mRNA for melatonin receptors was detected in P5 neopallium by RT-PCR (Husson et al., 2002). Furthermore, the 5-$HT_{2B/2C}$ receptor antagonist SB 206,533 was not protective indicating that the 5-HT_{2C} receptor antagonist facet of agomelatine's profile was not responsible for the neuroprotection.

The changes over time of the white matter lesion size indicate that both agomelatine and melatonin did not prevent the initial lesion but rather induced a secondary decrease of the white matter lesion size, suggesting some neurotrophic effect of these compounds. Although we cannot fully exclude the unlikely possibility that agomelatine and melatonin induces a rapid shrinkage of the cyst, previous studies using GAP-43 (growth cone-associated protein 43 kDa), Bodian and myelin basic protein staining argued for a melatonin-induced axonal regrowth or sprouting (Husson et al., 2002). Behavioral tests will be necessary to fully assess the functional consequence of this agomelatine and melatonin-induced neuroprotection. Furthermore, specific neurotrophic effects may well be important in some measures of antidepressant action (Agid et al., 2007).

The reason for agomelatine's larger time window of efficacy, as compared to melatonin, is not fully defined in these experiments. Agomelatine, but not melatonin, has robust antidepressant effects (Loo et al., 2002, 2003, Olié and Kasper, 2007) and it may be possible that the effects on neuroplasticity are linked (see Agid et al., 2007), but this will require a large research programme to establish. In addition, the protective effect of delayed administration of agomelatine was not only blocked by forskolin but also by PD98059, suggesting that this delayed neuroprotective effect of agomelatine requires the activation of the ERK pathway. It may well be that the delayed neuroprotective effects were mediated via the classical neurotrophic pathway, involving neurotrophins such as brain-derived neurotrophic factor (BDNF), which have already been implicated in neuroprotective effects of other dugs in this model. Thus S 18986, a positive modulator of AMPA receptors, was shown to increase BDNF levels and the protective effects of BDNF and S 18986 were blocked by PD98059, suggesting that this delayed neuroprotective effect of BDNF and S18986 required the activation of the ERK pathway (Dicou et al., 2003). BDNF, like melatonin and agomelatine, has delayed reparative effects on lesions in this model, and the time course is dose-dependent (Husson et al., 2005). Indirect protection mediated via neurotrophic factors may therefore be the most likely cause of the difference between melatonin and agomelatine. Furthermore neurotrophic factors have been implicated in the therapeutic effects of antidepressants (Manji et al., 2001).

The present study demonstrates that agomelatine and melatonin, both full agonists at melatonin receptors, display potent neuroprotective properties in a newborn mouse model of excitotoxic white matter lesions mimicking human periventricular leukomalacia. This model is predictive of neuroprotective and neurotrophic effects in physiopathological situations. Being a safe and well tolerated drug with demonstrated clinical efficacy in major depressive disorders (Loo et al., 2002, 2003, Olié and Kasper, 2007), agomelatine could therefore become part of the available therapeutic arsenal in the near future. If the present data can be extrapolated to the human perinatal situation, administration of agomelatine could become a very promising intervention in human pre-term neonates at high risk to develop brain lesions associated with cerebral palsy, for which there is major therapeutic need (Gressens and Spedding, 2004).

Acknowledgments

We are grateful to Jorge Gallego for the statistical analysis. This study is supported by the Inserm, Université Paris 7, Fondation Motrice, and Fondation Grace de Monaco.

References

Acarin, L., Gonzalez, B., Castro, A.J., Castellano, B., 1999. Primary cortical glial reaction versus secondary thalamic glial response in the excitotoxically injured young brain: microglial/macrophage response and major histocompatibility complex class I and II expression. Neuroscience 89, 549–565.

Agid, Y., Buzsáki, G., Diamond, D.M., Frackowiak, R., Giedd, J., Girault, J.A., Grace, A., Lambert, J.J., Manji, H., Mayberg, H., Popoli, M., Prochiantz, A., Richter-Levin, G., Somogyi, P., Spedding, M., Svenningsson, P., Weinberger, D., 2007. How can drug discovery for psychiatric disorders be improved? Nat. Rev. Drug. Discov. 6, 189–201.

Barks, J.D., Silverstein, F.S., 1992. Excitatory amino acids contribute to the pathogenesis of perinatal hypoxic-ischemic brain injury. Brain Pathol. 2, 235–243.

Bertaina-Anglade, V., Mocaer, E., Drieu la Rochelle, C., 2002. Anti-depressant-like action of S 20098 (agomelatine) in the learned helplessness test. Int. J. Neuropsychopharmacol. 5, S65.

Bromidge, S.M., Dabbs, S., Davies, D.T., Duckworth, D.M., Forbes, I.T., Ham, P., Jones, G.E., King, F.D., Saunders, D.V., Starr, S., Thewlis, K.M., Wyman, P.A., Blaney, F.E., Naylor, C.B., Bailey, F., Blackburn, T.P., Holland, V., Kennett, G.A., Riley, G.J., Wood, M.D., 1998. Novel and selective 5-HT2C/2B receptor antagonists as potential anxiolytic agents: synthesis, quantitative structure-activity relationships, and molecular modeling of substituted 1-(3-pyridylcarbamoyl)indolines. J. Med. Chem. 41, 1598–1612.

Conway, S., Canning, S.J., Howell, H.E., Mowat, E.S., Barrett, P., Drew, J.E., Delagrange, P., Lesieur, D., Morgan, P.J., 2000. Characterisation of human melatonin mt(1) and MT (2) receptors by CRE-luciferase reporter assay. Eur. J. Pharmacol. 390, 15–24.

Dammann, O., Leviton, A., 1997. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr. Res. 42, 1–8.

Dicou, E., Rangon, C.M., Guimiot, F., Spedding, M., Gressens, P., 2003. Positive allosteric modulators of AMPA receptors are neuroprotective against lesions induced by an NMDA agonist in neonatal mouse brain. Brain Res. 970, 221–225.

Dommergues, M.A., Patkai, J., Renauld, J.C., Evrard, P., Gressens, P., 2000. Proinflammatory cytokines and interleukin-9 exacerbate excitotoxic lesions of the newborn murine neopallium. Ann. Neurol. 47, 54–63.

Dommergues, M.A., Plaisant, F., Verney, C., Gressens, P., 2003. Early microglial activation following neonatal excitotoxic brain damage in mice: a potential target for neuroprotection. Neuroscience 121, 619–628.

Follett, P.L., Rosenberg, P.A., Volpe, J.J., Jensen, F.E., 2000. NBQX attenuates excitotoxic injury in developing white matter. J. Neurosci. 20, 9235–9241.

Gressens, P., Spedding, M., 2004. Strategies for neuroprotection in the newborn. Drug Discov. Today Ther. Strat. 1, 77–82.

Gressens, P., Marret, S., Hill, J.M., Brenneman, D.E., Gozes, I., Fridkin, M., Evrard, P., 1997. Vasoactive intestinal peptide prevents excitotoxic cell death in the murine developing brain. J. Clin. Invest. 100, 390–397.

Grether, J.K., Nelson, K.B., Emery 3rd, E.S., Cummins, S.K., 1996. Prenatal and perinatal factors and cerebral palsy in very low birth weight infants. J. Pediatr. 128, 407–414.

Hagberg, B., Hagberg, G., Olow, I., van Wendt, L., 1996. The changing panorama of cerebral palsy in Sweden. VII. Prevalence and origin in the birth year period 1987–90. Acta Paediatr. 85, 954–960.

Haynes, R.L., Folkerth, R.D., Keefe, R.J., Sung, I., Swzeda, L.I., Rosenberg, P.A., Volpe, J.J., Kinney, H.C., 2003. Nitrosative and oxidative injury to premyelinating oligodendrocytes in periventricular leukomalacia. J. Neuropathol. Exp. Neurol. 62, 441–450.

Husson, I., Mesples, B., Bac, P., Vamecq, J., Evrard, P., Gressens, P., 2002. Melatoninergic neuroprotection of the murine periventricular white matter against neonatal excitotoxic challenge. Ann. Neurol. 51, 82–92.

Husson, I., Rangon, C.M., Lelievre, V., Bemelmans, A.P., Sachs, P., Mallet, J., Kosofsky, B.E., Gressens, P., 2005. BDNF-induced white matter neuroprotection and stage-dependent neuronal survival following a neonatal excitotoxic challenge. Cereb. Cortex 15, 250–261.

Laudenbach, V., Calo, G., Guerrini, R., Lamboley, G., Benoist, J.F., Evrard, P., Gressens, P., 2001. Nociceptin/orphanin FQ exacerbates excitotoxic white-matter lesions in the murine neonatal brain. J. Clin. Invest. 107, 457–466.

Loeliger, M., Watson, C.S., Reynolds, J.D., Penning, D.H., Harding, R., Bocking, A.D., Rees, S.M., 2003. Extracellular glutamate levels and neuropathology in cerebral white matter following repeated umbilical cord occlusion in the near term fetal sheep. Neuroscience 116, 705–714.

Loo, H., Hale, A., D'Haenen, H., 2002. Determination of the dose of agomelatine, a melatoninergic agonist and selective 5-HT(2C) antagonist, in the treatment of major depressive disorder: a placebo-controlled dose range study. Int. Clin. Psychopharmacol. 17, 239–247.

Loo, H., Dalery, J., Macher, J.P., Payen, A., 2003. Pilot study comparing in blind the therapeutic effect of two doses of agomelatine, melatonin- agonist and selective 5HT2c receptors antagonist, in the treatment of major depressive disorders. Encephale 29, 165–171.

Manji, H.K., Drevets, W.C., Charney, D.S., 2001. The cellular neurobiology of depression. Nat. Med. 7, 541–547.

Marret, S., Mukendi, R., Gadisseux, J.F., Gressens, P., Evrard, P., 1995. Effect of ibotenate on brain development: an excitotoxic mouse model of microgyria and posthypoxic-like lesions. J. Neuropathol. Exp. Neurol. 54, 358–370.

McDonald, J.W., Shapiro, S.M., Silverstein, F.S., Johnston, M.V., 1998. Role of glutamate receptor-mediated excitotoxicity in bilirubin-induced brain injury in the Gunn rat model. Exp. Neurol. 150, 21–29.

Millan, M.J., Gobert, A., Lejeune, F., Dekeyne, A., Newman-Tancredi, A., Pasteau, V., Rivet, J.M., 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.

Olié, J.P., Kasper, S., 2007. Efficacy of agomelatine, a MT_1/MT_2 receptor agonist with 5-HT_{2C} antagonist properties, in major depressive disorder. Int. J. Neuropsychopharmacol. 10, 661–673.

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

Plaisant, F., Clippe, A., Vander Stricht, D., Knoops, B., Gressens, P., 2003. Recombinant peroxiredoxin 5 protects against excitotoxic brain lesions in newborn mice. Free Radic. Biol. Med. 34, 862–872.

Tahraoui, S.L., Marret, S., Bodenant, C., Leroux, P., Dommergues, M.A., Evrard, P., Gressens, P., 2001. Central role of microglia in neonatal excitotoxic lesions of the murine periventricular white matter. Brain Pathol. 11, 56–71.

Volpe, J.J., 2001. Neurobiology of periventricular leukomalacia in the premature infant. Pediatr. Res. 50, 553–562.

Yang, Q., Scalbert, E., Delagrange, P., Vanhoutte, P.M., O'Rourke, S.T., 2001. Melatonin potentiates contractile responses to serotonin in isolated porcine coronary arteries. Am. J. Physiol. Heart Circ. Physiol. 280, H76–H82.