THE NOVEL MELATONIN AGONIST, AGOMELATINE (S20098), IS AN ANTAGONIST AT 5-HYDROXYTRYPTAMINE$_{2C}$ RECEPTORS, BLOCKADE OF WHICH ENHANCES THE ACTIVITY OF FRONTOCORTICAL DOPAMINERGIC AND ADRENERGIC PATHWAYS

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

Dept of Psychopharmacology, Institut de Recherches Servier,
125 chemin de Ronde, 78290 Croissy/Seine, France

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Running Title: Monoamines and depression

Abbreviations:

ANOVA	Analysis of Variance
CHO	Chinese hamster ovary
DA	Dopamine
FCX	Frontal cortex
[^{35}S]GTPγS	Guanosine-5'-*O*-(3-[^{35}S]thio)-triphosphate
5-HT	Serotonin
LC	Locus coeruleus
NA	Noradrenaline
PI	Phosphatidylinositol
PLC	Phospholipase C
SPA	Scintillation-Proximity Assay
VTA	Ventrotegmental area

Dr Mark J. Millan, Institut de Recherches Servier, 125 chemin de Ronde
78290 Croissy/Seine, France
Tel: 33.1.55.72.24.26 - Fax: 33.1.55.72.24.70 - E-mail: mark.millan@fr.netgrs.com

Text pages	31
Tables	2
Figures	9
References	56
Abstract words	249
Introduction words	538
Discussion words	1544

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ABSTRACT

Agomelatine (S20098) displayed pK_is of 6.4 and 6.2 at native (porcine) and cloned, human (h)5-HT_{2C} receptors, respectively. It also interacted with h5-HT_{2B} receptors (6.6), whereas it showed low affinity at native (rat)/cloned human 5-HT_{2A} (<5.0/5.3) and 5-HT_{1A} (<5.0/5.2) receptors, and negligible (<5.0) affinity for other 5-HT receptors. In antibody-capture/Scintillation-Proximity-Assays, agomelatine concentration-dependently and competitively abolished h5-HT_{2C} receptor-mediated activation of Gq/11 and Gi_3 (pA_2s, 6.0 and 6.1). As measured by [^{3}H]phosphatidylinositol depletion, agomelatine abolished activation of phopholipase C by h5-HT_{2C} (pK_B, 6.1) and h5-HT_{2B} (pK_B, 6.6) receptors. *In vivo*, it dose-dependently blocked induction of penile erections by the 5-HT_{2C} agonists, Ro60,0175 and Ro60,0332. Further, agomelatine dose-dependently enhanced dialysis levels of dopamine in frontal cortex of freely-moving rats, whereas they were unaffected in nucleus accumbens and striatum. Though the electrical activity of ventrotegmental dopaminergic neurones was unaffected by agomelatine, it abolished their inhibition by Ro60,0175. Extracellular levels of noradrenaline in frontal cortex were also dose-dependently enhanced by agomelatine in parallel with an acceleration in the firing rate of adrenergic cell bodies in the locus coeruleus. These increases in noradrenaline and dopamine levels were unaffected by the selective melatonin antagonist, S22153, and likely reflect blockade of 5-HT_{2C} receptors inhibitory to frontocortical dopaminergic and adrenergic pathways. In distinction to agomelatine, melatonin showed negligible activity at 5-HT_{2C} receptors and failed to modify the activity of adrenergic and dopaminergic pathways. In conclusion, in contrast to melatonin, agomelatine behaves as an antagonist at 5-HT_{2B} and 5-HT_{2C} receptors: blockade of the latter reinforces frontocortical adrenergic and dopaminergic transmission.

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Melatonin, which is generated by the pineal gland, is an endogenous synchroniser of biological rhythms in mammals: its secretion and actions are tightly related to seasonal and light-dark cycles (Reppert et al., 1997; Borjigin et al., 1999). This chronobiotic role of melatonin has attracted considerable attention within the framework of depressive disorders inasmuch as they are aggravated by a disturbance of daily rhythms and sleep patterns (Souetre et al., 1989). Further, melatonin is active in several experimental models of antidepressant activity: notably, in a "chronic mild stress" paradigm in which diurnal and sleep rhythms are disrupted (Overstreet et al., 1998; Kopp et al., 1999). Though the relationship between melatonin levels and clinical depressive states is unclear, decreases have been reported (Szymanska et al., 2001; Tuunainen et al., 2002). Further, antidepressant treatment enhances melatonin levels reflecting actions of noradrenaline (NA) at pineal β-adrenoceptors facilitatory to its secretion (Borjigin et al., 1999; Skene et al., 1999; Szymanska et al., 2001). Melatonin acts *via* MT_1 and MT_2 receptors (Reppert et al., 1997; Borjigin et al., 1999), both of which control circadian rhythms (Liu et al., 1997). The novel agonist, agomelatine (S20098), possesses nanomolar affinity for MT_1 and MT_2 sites (Yous et al., 1992; Ying et al., 1998) and modulates circadian rhythms in rodents (Redman and Francis, 1998; Ying et al., 1998; Van Reeth et al., 2001). It is in Phase III clinical trials for the management of major depression and is active in certain rodent models predictive of antidepressant properties (Bourin et al., 2002; Papp et al., 2003), actions difficult to attribute *exclusively* to its engagement of MT_1/MT_2 receptors. Agomelatine possesses high overall selectivity for MT_1 and MT_2 receptors as compared to other sites (pK_i values of < 5.0 at > 50 sites, Cussac, D. et al., unpub. obs.). However, a binding “screen” suggested that it may interact with 5-HT_{2C} receptors. This observation is of considerable interest inasmuch as 5-HT_{2C} receptors are implicated in the etiology and treatment of depressive states.

Thus, 5-HT_{2C} receptors are enriched in the frontal cortex, hippocampus, basal ganglia and other structures implicated in the mood, motor and cognitive deficits which accompany depressive states (Sharma et al., 1997; Lopez-Gimenez et al., 2002). Many clinically-active antidepressant agents, such as mianserin, mirtazapine and amitryptiline, behave as antagonists at 5-HT_{2C} receptors (Jenck et al., 1994; Millan et al., 2000a), while long-term administration of 5-HT reuptake inhibitors results in their down-regulation (Bristow et al., 2000). Notably, in contrast to their 5-HT_{2A} and 5-HT_{2B} counterparts, 5-HT_{2C} receptors exert a tonic, inhibitory influence upon frontocortical dopaminergic and adrenergic pathways, the activity of which is compromized in depressive states (Di Giovanni et al., 1999; Gobert et al., 2000; Millan et al., 2000b). Indeed, *via* contrasting mechanisms, a common property of all clinically-active antidepressant agents may be an increase in extracellular levels of noradrenaline (NA) and dopamine (DA) in the frontal cortex (FCX) of rodents (Millan et al., 2000b).

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In light of the above observations, the present studies had two major and interrelated aims. *First*, to characterize the interaction of agomelatine with 5-HT_{2C} receptors both *in vitro* and *in vivo* and, *second*, to examine its influence upon ascending monoaminergic pathways which, as outlined above, are subject to a tonic, inhibitory control by 5-HT_{2C} receptors. In all procedures, the actions of agomelatine were compared to those of melatonin.

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METHODS

Animals. *In vivo* studies employed male Wistar rats (225-250 g for dialysis studies, and 120-140 g for behavioral studies). They were housed in sawdust-lined cages with unrestricted access to food and water. Laboratory humidity was 60 ± 5 % and temperature 21 ± 1 °C. Lights were on from 7:30 h to 19:30 h. Animals were adapted for at least 1 week to laboratory conditions prior to use. All animal use procedures conformed to international european ethical standards (86/609-EEC) and the French National Commitee (décret 87/848) for the care and use of laboratory animals.

Competition binding assays. Binding affinities at h5-HT_{2A}, h5-HT_{2B} and h5-HT_{2C} receptors stably transfected into Chinese hamster ovary (CHO) cells were determined essentially as described (Cussac et al., 2002b) by competition binding with [^{3}H]ketanserin (0.5 nM, Amersham, Les Ulis, France) (h5-HT_{2A} receptors) or [^{3}H]mesulergine (1 nM, Amersham, Les Ulis, France) (h5-HT_{2B} and h5-HT_{2C} receptors) in a buffer containing HEPES (20 mM) pH 7.5, EDTA (2 mM) and ascorbic acid (0.1% w/v). Incubations lasted 2 h at 22 °C and non-specific binding was defined by 5-HT (10 µM) for h5-HT_{2B} receptors and mianserin (10 µM) for h5-HT_{2A} and h5-HT_{2C} receptors. Binding affinities at native, rat 5-HT_{2A} (frontal cortex) and porcine 5-HT_{2C} receptors (choroid plexus) were determined by standard techniques employing [^{3}H]ketanserin (0.5 nM) and [^{3}H]mesulergine (1.0 nM), respectively, as radioligands (Millan et al., 2000a). Binding affinities at 5-HT_{1A} receptors in membranes from rat hippocampus and from CHO cells stably expressing recombinant h5-HT_{1A} receptors (PerkinElmer Life Sciences, Boston, MA) were determined essentially as described (Newman-Tancredi et al., 1997) by competition binding with [^{3}H]8-OH-DPAT (0.4 nM, Amersham, Les Ulis, France). Non-specific binding was defined using 5-HT (10 µM). Experiments were terminated by rapid filtration through Unifilter-96 GF/B filters (pretreated with polyethyleneimine 0.1%) using a Filtermate harvester (PerkinElmer Life Science, Boston, MA). Radioactivity retained on the filters was determined by liquid scintillation counting using a Top-Count microplate scintillation counter (PerkinElmer Life Science). Non-linear, regression analysis of isotherms was undertaken by Prism software (Graphpad Software, San Diego, CA) to yield Inhibitory Concentration 50 (IC_{50}) values. These were transformed into K_i values according to Cheng-Prussof: $K_i = IC_{50}/(1 + L/K_D)$, where L corresponds to the radioligand concentration and K_D its dissociation constant.

Activity at h5-HT_{2C} receptors coupled to Gq/11 and Gi_3: Scintillation Proximity Assays. Most studies of coupling at 5-HT_{2C} receptors have focussed on their stimulation of Gq/11 (Gerhardt and Heerikhuizen, 1997; Cussac et al., 2002a). However, they can also engage the Gi protein family (Alberts et al., 1999; Cussac et al., 2002a). Inasmuch as individual ligands

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differentially influence specific G-proteins by "agonist-directed trafficking" (Berg et al., 1998), we determined the influence of agomelatine upon both Gq/11 and Gi_3 employing novel, antibody-capture based Scintillation Proximity Assays (SPA) (Cussac et al., 2002a). The stimulation of guanosine-5'-*O*-(3-[^{35}S]thio)-triphosphate ([^{35}S]-GTPγS) (1332 Ci/mmol, NEN, Paris, France) binding at Gq/11 and Gi_3 was measured as previously described (Cussac et al., 2002a). CHO-h5-HT_{2C} membranes (~20-30 µg per well) were pre-incubated for 30 min with agomelatine (or melatonin) with or without 5-HT in a buffer containing HEPES 20 mM (pH 7.4), GDP 0.1 µM, $MgCl_2$ 50 mM and NaCl 150 mM. The reaction was started with [^{35}S]-GTPγS (0.2 nM) in a final volume of 200 µl in 96-well plates for 60 min at room temperature. Specific activation of different subtypes of G-protein was determined using a SPA approach as described by DeLapp et al. (1999) and Cussac et al. (2002a). At the end of the incubation period, 20 µl of NP40 (0.27 % final concentration) was added to each well and the plates incubated with gentle agitation for 30 min. Antibodies specific for Gq/11 (1.74 µg/ml, final dilution) and Gi_3 (0.87 µg/ml, final dilution) were added to each well in a volume of 10 µl before 30 min of additional incubation. SPA beads coated with secondary anti-rabbit or anti-mouse antibodies (Amersham, Little Chalfont, UK) were added and the plates incubated for 3 h with gentle agitation. The plates were then centrifuged (10 min, 1,300 g) and radioactivity detected on a Packard 'Top-count' microplate scintillation counter. Isotherms were analysed by non-linear regression using the program 'PRISM' (Graphpad Software Inc., San Diego, CA) to yield Effective Concentrations (EC_{50}) and Inhibition Concentrations (IC_{50}) values. K_B values of agomelatine and melatonin for inhibition of 5-HT-stimulated [^{35}S]-GTPγS binding were calculated as previously (Cussac et al., 2002a): $K_B = IC_{50} / (1+(Agonist / EC_{50}))$, where IC_{50} = Inhibitory $Concentration_{50}$ of antagonist, Agonist = concentration of 5-HT and EC_{50} = Effective $Concentration_{50}$ of 5-HT alone. In additional antagonist studies, the concentration-response curve for 5-HT was performed in the presence of incremental concentrations of agomelatine and Schild analyses undertaken to yield pA_2 values (Arunlakshana and Schild, 1956).

Influence upon h5-HT_{2C} and h5-HT_{2B} receptor-mediated [^{3}H]PI depletion. *Via* Gq/11, -HT_{2C} receptors couple to Phospholipase C (PLC), activation of which generates cytosolic inositol phosphates by the conversion of membrane-bound phosphatidylinositols (PI), the principle substrate of PLC. Thus, employing a model of [^{3}H]PI depletion (Cussac et al., 2002b), we evaluated the influence of agomelatine upon the activity of PLC. Phospholipase C activity was monitored as previously described (Cussac et al., 2002b) by measuring the drug-induced decrease of [^{3}H]PI levels in transfected CHO membranes. Drug efficacies were expressed relative to that of a maximally-effective concentration of 5-HT (1 µM, defined as 100 %). For antagonist studies, concentration-response curves of agomelatine (and melatonin) against 5-HT were analysed as described above to yield K_B values. In an additional antagonist

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study of h5-HT_{2C} receptors, the concentration-response curve for 5-HT was performed in the presence of incremental concentrations of agomelatine and Schild analyses performed to yield pA_2 values.

Antagonist properties at 5-HT_{2C} receptors *in vivo*: blockade of Ro60,0175- and Ro60,0332-induced penile erections. Amongst several *in vivo* responses attributed to 5-HT_{2C} receptors, the induction of penile erections is particularly well-defined (Bos et al., 1998; Millan et al., 1997). Accordingly, to confirm the actions of agomelatine at 5-HT_{2C} receptors *in vivo*, we examined the influence of agomelatine upon their induction by the high efficacy 5-HT_{2C} ligands, Ro60,0175 and Ro60,0332 (Bos et al., 1998; Cussac et al., 2002a,b). As described previously (Millan *et al*., 1997), rats were individually placed in transparent, plexiglass observation cages immediately following drug or vehicle administration. Thirty min later, the animals received Ro60,0175 (1.25 mg/kg, s.c.) or Ro60,0332 (2.5 mg/kg, s.c.) and penile erections were measured over a 30 min observation period.

Influence upon dialysate levels of monoamines in the FCX, nucleus accumbens and striatum of freely-moving rats. Finally, we employed a combined dialysis and electrophysiological approach (Gobert et al., 2000) for characterization of the influence of agomelatine upon the activity of frontocortical dopaminergic and adrenergic pathways.The protocol used for quantification of DA, NA and 5-HT levels in single dialysate samples of FCX, nucleus accumbens and striatum of freely-moving rats has been detailed elsewhere (Gobert *et al*., 2000). Guide cannulae were implanted in rats under pentobarbital anaesthesia (60 mg/kg, i.p.) at the following coordinates: FCX, AP = + 2.2 from bregma, L = ± 0.6 and H = - 0.2 from dura; nucleus accumbens, AP = + 0.8 from bregma, L = + 0.6 and H = - 4.5 from dura and striatum, AP = + 0.5 from bregma, L = - 2.8 and H = - 3.0 from dura. The nucleus accumbens and striatum were examined ("dual probe") simultaneously. All dialysis experiments were performed 5 days after placement of guide cannulae. A cuprophane CMA/11 probe (4 mm in length for the FCX and striatum, 2 mm in length for the nucleus accumbens and, in each case, 0.24 mm of outer diameter) was lowered into position and perfused at 1 µl/min with a phosphate-buffered solution of NaCl: 147.2 mM; KCl: 4 mM; $CaCl_2$: 2.3 mM (pH, 7.3). Two hours after implantation, samples (20 µl) were taken every 20 min and, following 3 basal samples, agomelatine (2.5 - 80.0 mg/kg, i.p.), melatonin (40.0 mg/kg, i.p.) or vehicle were injected and sampling pursued for 3 hr. In the antagonist study, S22153 (a selective melatonin antagonist) was injected (20.0 mg/kg, i.p.) 20 min prior to agomelatine (40.0 mg/kg, i.p.). The influence of drugs and vehicle was expressed relative to basal values (defined as 100 %). The assay sensitivity was ca 0.1-0.2 pg/sample for DA, NA and 5-HT in each case.

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Modulation of the electrical activity of dopaminergic and adrenergic neurones in anaesthetised rats. Techniques detailed previously (Gobert et al., 2000; Millan et al., 2000a) were employed for determination of the influence of drugs upon the electrical activity of dopaminergic and adrenergic perikarya localised in the ventrotegmental area (VTA) and locus coeruleus (LC), respectively. Rats were anaesthetised with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic apparatus. A tungsten microelectrode was lowered into the VTA or LC. Coordinates were as follows: VTA, AP = 5.5 from bregma, L = 0.7, H = -7/-8.5 from dura and LC, AP = -1.2 from zero, L = 1.2 and H = -5.5/-6.5 from the sinus surface. As in our previous studies (Gobert et al., 2000; Millan et al., 2000a), dopaminergic and adrenergic neurones were identified according to their waveforms, firing rhythms and response to selective agonists at dopamine D_2/D_3 and α_2-adrenoceptors, respectively (Aghajanian and Bunney, 1973; Aghajanian et al 1977; Wang, 1981; Chiang and Aston-Jones, 1993). Briefly, dopaminergic neurons in the VTA displayed triphasic potentials (+/-/+) of > 3 msec duration with a notch on the ascending limb: they showed a firing rate of 2 to 8 Hz, primarly in bursts. Adrenergic neurons in the LC showed and biphasic potentials (+/-) of > 2 msec duration with a notched wave form on the final ascending limb and a constant, tonic, firing rate of 0.5 to 3 Hz. Further, in response to contralateral paw pinch, they revealed a brief acceleration of firing (burst) followed by a transient silent period. Baseline recording was undertaken for at least 5 min. One cell was recorded in each animal. The influence of agomelatine (1.0 - 16.0 mg/kg, i.v.) as compared to melatonin (16.0 mg/kg, i.v.) and vehicle (1/10 ethanol + 4/10 polyethyleneglycol 400 + 5/10 sterile water, injected i.v. in a volume of 0.5 ml/kg) upon firing rate was evaluated by administration in cumulative doses at intervals of 2-3 min. For antagonist studies with the 5-HT_{2C} agonist, Ro60,0175 (1.0 mg/kg, i.v.), agomelatine (4.0 mg/kg, i.v.) and melatonin (4.0 mg/kg, i.v.) were administered 2-3 min following its administration. Following completion of studies of the VTA and LC, the D_2/D_3 agonist, apomorphine (0.031 mg/kg, i.v.), or the α_2-adrenoceptor agonist, clonidine (0.01 mg/kg, i.v.), were injected to further confirm the identity of dopaminergic and adrenergic neurons, respectively.

Drugs. With the exception of electrophysiological studies (see above), agomelatine, melatonin and S22153 were administered i.p. as suspensions (in a few drops of Tween 80) in distilled water. Apomorphine and clonidine were injected i.v., and Ro60,0175 and Ro60,0332 were administered s.c. They were dissolved in sterile water plus a few drops of lactic acid and the pH adjusted with NaOH to as close to neutrality as possible (pH > 5.0). Drugs injected i.p. and s.c. were given in a volume of 1 ml/kg body weight. Doses refer to the base. Drug salts, structures and sources were as follows: Apomorphine HCl, clonidine HCl and melatonin base were obtained from Sigma, St Quentin-Fallavier, France and agomelatine base, S22153 base (N-[2-(5-ethyl-benzo[b]thien-3-yl)ethyl] acetamide), Ro60,0175 fumarate ((S)-2-(6-chloro-5-

fluoroindol-1-yl)-1-methylethylamine)) and Ro60,0332 fumarate (1-methyl-2-(5,8,8-trimethyl-8H-3-aza-cyclopenta[a]inden-3-yl) ethylamine) were synthetized by Servier chemists.

Data Analysis and Statistics. Dose-response curves from *in vivo* studies were analysed by Analysis of Variance (ANOVA) followed, as appropriate, by Dunnett's test or Newman-Keuls test. Single-dose comparisons were analysed employing Student's two-tailed t-test.

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RESULTS

Binding profile of agomelatine and melatonin (Table 1). Agomelatine monophasically displaced the binding of [^{3}H]mesulergine to h5-HT$_{2C}$ receptors, whereas melatonin exerted little influence over a similar range of concentrations. Similarly, agomelatine displayed higher affinity for native, porcine 5-HT$_{2C}$ receptors than melatonin. At h5-HT$_{2B}$ receptors, agomelatine also more potently displaced the binding of [^{3}H]mesulergine than melatonin. The affinities of agomelatine and melatonin were comparatively low for both h5-HT$_{2A}$ and native, rat 5-HT$_{2A}$ receptors. Agomelatine and melatonin displayed negligible affinities for native, rat 5-HT$_{1A}$ receptors (pK_is, < 5.0) and low affinities for cloned, h5-HT$_{1A}$ receptors (pK_is, 5.25 and 5.31, respectively). Employing standard binding protocols, moreover, agomelatine failed to significantly bind to all other 5-HT receptors examined (h5-HT$_{1B}$, h5-HT$_{1D}$, cloned, murine 5-HT$_3$, h5-HT$_4$, h5-HT$_{5A}$, h5-HT$_6$ and h5-HT$_7$): pK_i values in each case of < 5.0 with < 10 % displacement of radioligand at a concentration of agomelatine of 10 µM (not shown). Likewise, agomelatine showed negligible affinity for cloned, human and native, rat 5-HT, NA and DA transporters: pK_i values in each case of < 5.0 with < 10 % displacement of radioligand at a concentration of agomelatine of 10 µM (not shown).

Antagonist properties of agomelatine and melatonin at h5-HT$_{2C}$ receptors: SPA analysis (Figs 1 and 2 and Table 2). Agomelatine and melatonin did not activate Gq/11 and Gi$_3$ proteins when tested alone. While melatonin failed to block stimulation of Gq/11 and Gi$_3$ proteins by 5-HT, agomelatine concentration-dependently and completely blocked 5-HT-induced Gq/11 and Gi$_3$ protein activation with pK_Bs of 6.0 and 5.9, respectively. In the presence of incremental concentrations of agomelatine, the concentration-response curves for 5-HT-induced Gq/11 and Gi$_3$ activation were displaced in parallel to the right without a loss of maximal effect. These data generated linear Schild plots with slopes not significantly different from unity (0.96 for Gq/11, and 0.94 for Gi$_3$), yielding pA_2 values of 6.0 for both Gq/11 and Gi$_3$.

Antagonist properties of agomelatine and melatonin at h5-HT$_{2C}$ receptors: [^{3}H]PI depletion (Fig 3 and Table 2). Neither agomelatine nor melatonin led to [^{3}H]PI depletion when tested alone. However, while melatonin did not block the action of 5-HT (10 nM), agomelatine concentration-dependently and completely blocked 5-HT-induced [^{3}H]PI depletion with a pK_B of 6.1. In the presence of incremental concentrations of agomelatine, the concentration-response for 5-HT was displaced in parallel to the right with no loss of maximal effect. Though the curve tended to flatten at the highest concentration of agomelatine, the slope derived from Schild analysis was not different to unity (0.94). Further, the pA_2 of 6.1 corresponded well to the pK_B.

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Antagonist properties of agomelatine and melatonin at h5-HT$_{2B}$ receptors: [^{3}H]PI depletion (Fig 4 and Table 2). Agomelatine failed to elicit [^{3}H]PI depletion alone and concentration-dependently blocked the action of 5-HT with a pK_b of 6.6 corresponding well to its pK_i (6.6) at these sites. Melatonin likewise did not enhance [^{3}H]PI depletion and partially attenuated the action of 5-HT, though only ~50 % of inhibition was acquired even at a concentration of 100 μM. It was not possible, for reasons of solubility, to evaluate higher concentrations of melatonin.

Antagonist properties of agomelatine and melatonin: Inhibition of penile erections evoked by 5-HT$_{2C}$ agonists (Fig 5). In agreement with our previous investigation (Millan et al., 1997), the high efficacy 5-HT$_{2C}$ agonist, Ro60,0175 (Bos et al., 1998; Cussac et al., 2002a,b), elicited a marked penile erection response in rats (0.16 - 2.5 mg/kg, s.c.) Agomelatine, which did not itself elicit penile erection (0.63 and 40.0 mg/kg, i.p.) concentration-dependently (2.5 - 80.0 mg/kg, i.p.) blocked the action of Ro60,0175 (2.5 mg/kg, s.c.). A further high efficacy 5-HT$_{2C}$ agonist of greater selectivity, Ro60,0332 (Bos et al., 1998; Cussac et al., 2002a,b), similarly evoked penile erections (0.16 - 2.5 mg/kg, s.c.) and its actions (2.5 mg/kg, s.c.) were also dose-dependently (2.5 - 40.0 mg/kg, i.p.) abolished by agomelatine. Melatonin (10.0 and 40.0 mg/kg, i.p.) neither evoked penile erections (10.0 and 40.0 mg/kg, i.p.) nor significantly interfered (2.5 - 40.0 mg/kg, i.p.) with their induction by Ro60,0175 and Ro60,0332. ANOVA as follows. For induction of penile erections, Ro60,0175, $F(4,42) = 15.4$, $P < 0.001$; Ro60,0332, $F(4,39) = 10.4$, $P < 0.001$; agomelatine, $F(2,21) = 0.5$, $P > 0.05$ and melatonin, $F(2.21) = 1.0$, $P > 0.05$. For blockade of the actions of Ro60,0175, agomelatine, $F(4,43) = 21.1$, $P < 0.001$ and melatonin, $F(2,21) = 1.0$, $P > 0.05$, and for blockade of the actions of Ro60,0332, agomelatine, $F(4,56) = 4.5$, $P < 0.01$ and melatonin, $F(4,43) = 0.7$, $P > 0.05$.

Influence of agomelatine and melatonin upon extracellular levels of DA and NA in the FCX as compared to the nucleus accumbens and striatum of freely-moving rats (Fig 6). Administration of vehicle elicited a transient elevation in FCX levels of NA (but not DA) which has previously been shown to reflect handling and manipulation (see Millan et al., 2000b). In comparison, agomelatine (2.5 - 80.0 mg/kg, i.p.) elicited a marked, sustained and dose-dependent elevation in extracellular levels of both DA and NA in the FCX. Quantified in the same samples, there was no alteration in levels of 5-HT (not shown). ANOVA as follows. DA: agomelatine (2.5), $F(1,9) = 1.1$, $P > 0.05$; agomelatine (10.0), $F(1,10) = 7.8$, $P < 0.05$; agomelatine (20.0), $F(1,11) = 27.8$, $P < 0.01$; agomelatine (40.0), $F(1,10) = 37.2$, $P < 0.01$ and agomelatine (80.0), $F(1,10) = 15.1$, $P < 0.01$ and NA, agomelatine (2.5), $F(1,9) = 0.1$, $P > 0.05$; agomelatine (10.0), $F(1,10) = 0.3$, $P > 0.05$; agomelatine (20.0), $F(1,11) = 4.5$, $P <$

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0.05; agomelatine (40.0), F (1,10) = 13.0, P < 0.01 and agomelatine (80.0), F (1,10) = 13.8, P < 0.01. Melatonin (40.0 mg/kg, mg/kg, i.p., n = 7) did not, in contrast to agomelatine, significantly modify levels of DA, NA or (not shown) 5-HT. Area-under-the-curve analysis as follows: For DA, vehicle = 106.4 ± 5.0 % *versus* melatonin = 118.3 ± 4.8 % (P > 0.05) and for NA, vehicle = 118.6 ± 5.1 % *versus* melatonin = 135.9 ± 6.5 % (P > 0.05). Administered at a dose (40.0 mg/kg, i.p., n = 6) which elicited a pronounced increase in extracellular levels of DA in the FCX, agomelatine did not significantly modify dialysis levels of DA in terminal regions of subcortical projections, the nucleus accumbens and the striatum. ANOVA as follows. Nucleus accumbens, agomelatine (40.0), F (1,10) = 0.4, P > 0.05 and striatum, DA: agomelatine (40.0), F (1,10) = 0.1, P > 0.05. Serotonin levels were also unaffected by agomelatine in these structures (not shown). Melatonin (40.0 mg/kg, i.p., n = 7) likewise did not affect levels of DA or 5-HT (not shown) in the nucleus accumbens and striatum. Area-under-the-curve analysis as follows (for DA): For the nucleus accumbens, vehicle = 97.5 ± 2.4% *versus* melatonin = 91.7 ± 2.0 % (P > 0.05) and for the striatum, vehicle = 91.6 ± 4.1 % *versus* melatonin = 79.8 ± 3.1 % (P > 0.05).

Lack of influence of the selective melatonin antagonist, S22153, upon the increase in extracellular levels of NA and DA elicited by agomelatine in the FCX of freely-moving rats (Fig 7). Administration of the selective melatonin (MT_1/MT_2) antagonist, S22153 (Weibel et al., 1999), at a dose of 20.0 mg/kg, i.p., did not itself influence levels of DA or NA in FCX dialysates. In its presence, the facilitatory influence of agomelatine (40.0 mg/kg, i.p.) upon the release of DA and NA was not modified. ANOVA as follows. DA: influence of agomelatine: F (1,9) = 78.1, P < 0.01; influence of S22153, F (1,10) = 0.6, P > 0.05 and interaction, F (1,8) = 0.1, P > 0.05. NA: influence of agomelatine, F (1,9) = 19.8, P < 0.01; influence of S22153, F (1,9) = 0.4, P > 0.05 and interaction, F (1,8) = 0.1, P > 0.05. S22153 also failed to affect levels of 5-HT, either alone or when co-administered with agomelatine (not shown).

Influence of agomelatine and melatonin upon the electrical activity of adrenergic neurones (Fig 8). In anesthetized rats, as originally characterized elsewhere (Aghajanian et al, 1977; Chiang and Aston-Jones, 1993), and in line with our previous studies (Gobert et al., 2000; Millan et al., 2000a), adrenergic neurones in the LC could be recognized by: their distinctive notched and biphasic waveform (> 2 Msec), their slow tonic firing rate, their transient acceleration upon "pinching" the contralateral hind-paw and, following evaluation of the test drug, the complete inhibition of their firing rate by administration of the α_2-adrenoceptor agonist, clonidine. A representative spike tracing is presented in Fig 8. Administered by the i.v. route, agomelatine (1.0 - 16.0 mg/kg, i.v.) elicited a dose-dependent and pronounced increase in the firing rate of adrenergic perikarya. ANOVA as follows, F

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(5,29) = 4.15, P < 0.01. In all neurones responsive to agomelatine, subsequent administration of clonidine (0.01 mg/kg, i.v.) abolished their electrical activity. In contrast to agomelatine, melatonin (16.0 mg/kg, i.v.) did not significantly modify the electrical activity of adrenergic cell bodies.

Influence of agomelatine and melatonin upon the electrical activity of dopaminergic neurones (Fig 9). In anesthetized rats, as originally characterized by others (Aghajanian and Bunney, 1973; Wang, 1981), and in line with our previous studies (Gobert et al., 2000; Millan et al., 2000a), dopaminergic neurones in the VTA were identified by their characteristic, long-lasting (> 3Msec) triphasic waveform, their primarily burst firing mode and, following evaluation of the test drug, inhibition of their activity by the D_2/D_3 dopamine receptor agonist, apomorphine. A representative spike tracing is presented in Fig 9. In contrast to adrenergic perikarya, agomelatine (1.0 - 16.0 mg/kg, i.p.) did not significantly modify the firing rate of dopaminergic neurones: further, it did not change the ratio of regular to burst firing (not shown). The 5-HT_{2C} agonist, Ro60,0175 (1 mg/kg, i.v.), markedly reduced the firing rate of dopaminergic perikarya, corroborating our previous study (Gobert et al., 2000). This inhibitory influence of Ro60,0175 was reversed by agomelatine (4 mg/kg, i.v.). ANOVA as follows: influence of Ro60,0175, F (1,22) = 75.6, P < 0.001; influence of agomelatine, F (1,22) = 15.8, P < 0.01 and influence of melatonin, F (1,22) = 0.2, P > 0.05. Melatonin (4 mg/kg, i.v.) neither affected the basal firing rate of dopaminergic neurones nor modified their response to Ro60,0175. The electrical activity of neurones was, in all cases, abolished by apomorphine (0.031 mg/kg, i.v.).

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DISCUSSION

Interaction of agomelatine with 5-HT$_2$ receptors *versus* other classes of 5-HT receptor. Agomelatine could be distinguished from melatonin by its interaction with 5-HT$_{2C}$ and 5-HT$_{2B}$ receptors, though its affinity for 5-HT$_{2A}$ and other serotonergic 5-HT sites was low. Notably, agomelatine also showed low affinity for h5-HT$_{1A}$ receptors and, even at 10 µM, neither stimulated nor blocked h5-HT$_{1A}$ sites in a GTPγS binding assay (Cussac, D, unpub. obs.). Correspondingly, while raphe-localized serotonergic neurones bear 5-HT$_{1A}$ autoreceptors, agomelatine influenced neither their firing rate nor dialysate levels of 5-HT (*vide infra*).

Antagonist actions of agomelatine at h5-HT$_{2C}$ receptors *in vitro*. 5-HT induces [^{35}S]GTPγS binding to both Gq/11 and Gi$_3$ *via* h5-HT$_{2C}$ receptors, actions blocked by the selective 5-HT$_{2C}$ antagonist, SB242,084 (Cussac et al., 2002a). By analogy, agomelatine abolished stimulation of Gq/11 and Gi$_3$ by 5-HT, whereas melatonin was inactive. The dextral displacement of the concentration-response curve for 5-HT by agomelatine indicates that it behaves as a competitive antagonist. Underpinning this contention, for both Gq/11 and Gi$_3$, pA$_2$ values corresponded well to pK$_B$ and pK$_i$ values. That agomelatine acts as a pure antagonist at two different G-protein subtypes coupled to h5-HT$_{2C}$ receptors is important inasmuch as: 1), they may control different functions *in vivo* and 2), certain 5-HT$_{2C}$ receptor ligands differentially influence G protein subtypes (Berg et al., 1998; Cussac et al., 2002a). Agomelatine similarly behaved as a competitive antagonist in blocking 5-HT-elicited [^{3}H]PI depletion, presumably reflecting activation of PLC by Gq/11 (Gerhardt and Herrekuiken, 1997). The lack of intrinsic actions of agomelatine underscores its antagonist profile inasmuch as (reflecting amplification between G-proteins and PLC) [^{3}H]PI depletion is a particularly sensitive measure of efficacy (Gerhardt and Herrekuiken, 1997; Cussac et al., 2002b).

These studies were undertaken with the "VSV" (edited) isoform of h5-HT$_{2C}$ receptors which is broadly distributed in cerebral tissues (Herrick-Davis et al., 1999). As compared to the VSV isoform, wild-type ("INI") 5-HT$_{2C}$ receptors display constitutive activity: "neutral" antagonists behave as inverse agonists (Berg et al., 1998; Herrick-Davis et al., 1999). Though the pathophysiological significance of constitutive activity remains unclear, potential inverse agonist actions of agomelatine at 5-HT$_{2C}$ receptors would be of interest to examine**.**

Antagonist properties of agomelatine at 5-HT$_{2C}$ receptors *in vivo*. The 5-HT$_{2C}$ receptor agonists, Ro60,0175 and Ro60,0332, elicit penile erections in rats, a response abolished by antagonists at 5-HT$_{2C}$, but not 5-HT$_{2A}$ or 5-HT$_{2B}$, receptors (Millan et al., 1997; Bos et al., 1998). Blockade of their actions by agomelatine indicates that, in distinction to melatonin, it

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behaves as an antagonist at native 5-HT_{2C} receptors *in vivo*, underpinning cellular studies. The relevance of antagonist actions in this model to sexual behaviour remains unclear. Antidepressants possessing antagonist properties at 5-HT_{2C} receptors, such as mirtazapine, elicit *less* problems of diminished libido than drugs which increase extracellular levels of 5-HT: indeed, the suppressive influence of 5-HT uptake inhibitors upon sexual behaviour involves *activation* of 5-HT_{2C} receptors (Rosen et al., 1999; Gelenberg et al., 2000).

The affinity of agomelatine at 5-HT_{2C} receptors is substantially (> 100-fold) lower than at MT_1 and MT_2 receptors. Correspondingly, in comparison to chronobiotic paradigms reflecting engagement of melatonin sites (Armstrong et al., 1993; Redman et al., 1998; Van Reeth et al., 2001), agomelatine exerts actions at 5-HT_{2C} receptors (this paper and Dekeyne, A. unpub. obs.) over a higher dose-range. In fact, the degree of dose-separation (~10-20 fold) is *less* pronounced than expected from its differential affinities. This may reflect the difficulty of comparing potencies between agonist actions at one site (MT) and *antagonist* actions at another (5-HT_{2C}). Further, agomelatine may be a more potent antagonist at 5-HT_{2C} isoforms other than VSV *(vide supra*) (Berg et al., 1998). In any case, these *in vivo* studies show that the modest affinity of agomelatine *is* sufficient to block cerebral 5-HT_{2C} receptors. In support of this assertion, plasma levels of agomelatine (12.8 µM at 40.0 mg/kg, s.c., 1 hour following administration) are *superior* to its affinity (1 µM) for these sites. Whether 5-HT_{2C} sites are involved in clinical actions of agomelatine (Loo et al, 2002) remains to be ascertained.

Antagonist actions of agomelatine at h5-HT_{2B} receptors *in vitro*. The binding and coupling profiles of h5-HT_{2B} and h5-HT_{2C} receptors are similar (Gerhardt and Heerikhuizen, 1997; Cussac et al., 2002b) and agomelatine also blocked 5-HT-induced [^{3}H]PI depletion at h5-HT_{2B} sites. 5-HT_{2B} receptors are poorly represented in the CNS wherein their functional significance remains obscure (Duxon et al., 1997). Indeed, studies specifically examining their potential role in the induction of penile erections and modulation of monoaminergic transmission have yielded negative findings (Millan et al., 1997; 2000b; Bos et al., 1998; Di Giovanni et al, 1999; Gobert et al., 2000; Di Matteo et al., 2001). Though their engagement reduces anxiety and elicits hyperphagia in rats (Kennett et al., 1998), like selective 5-HT_{2B} receptor antagonists, agomelatine shows neither anxiogenic nor appetite-suppressant properties (Dekeyne, A. unpub. obs.). Further, while stimulation of 5-HT_{2B} sites exerts actions in peripheral tissues - in particular in development - there is no evidence for functional effects of *antagonists* at these sites (Nebigil and Maroteaux, 2001).

Lack of antagonist actions of agomelatine at 5-HT_{2A} receptors. Though the low affinity of agomelatine for h5-HT_{2A} sites precluded antagonist studies, it failed to trigger [^{3}H]PI depletion at high concentrations (10^{-4} M). Further, agomelatine (80.0 mg/kg, s.c.) neither

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increased levels of corticosterone nor modified their elevation by 5-HT_{2A} agonists (Rivet, J-M. and Millan, M.J., unpub. obs). Thus, agomelatine does *not* exert actions *via* 5-HT_{2A} receptors. Extending a report of Dugovic et al. (1989) at rat 5-HT_{2A} sites, melatonin had low affinity for h5-HT_{2A} receptors.

Modulation by agomelatine of frontocortical dopaminergic and adrenergic transmission. Agomelatine elevated extracellular levels of NA and DA in FCX whereas, in line with its low affinity for 5-HT_{1A} sites, levels of 5-HT were *unaffected*. Accordingly, though activation of 5-HT_{1A} autoreceptors disinhibits frontocortical catecholaminergic transmission (Millan et al, 2000), this mechanism cannot be involved in the elevation in FCX levels of NA and DA by agomelatine. Further, this action of agomelatine was insensitive to the melatonin (MT_1/MT_2) antagonist, S22153, at a dose which abolishes its *in vivo* actions at melatoninergic receptors (Weibel et al., 1999; Ying et al., 1999). The inability of melatonin to elevate levels of DA and NA in FCX also suggests that the agonist actions of agomelatine at melatoninergic receptors are not involved in its reinforcement of catecholaminergic input to the FCX. (Interestingly, melatonin *inhibits* DA release in the hypothalamus (Zisapel, 2002)). Contrariwise, several arguments support a role of 5-HT_{2C} receptor blockade in the induction of frontocortical release of NA and DA by agomelatine.

First, the magnitude of its effect was comparable to that of the 5-HT_{2C} antagonists, SB206,553 and SB242,084 (Gobert et al., 2000). *Second*, the active dose-range for agomelatine corresponds well to that which blocks induction of penile erections by Ro60,0175 and Ro60,0332. *Third*, though agomelatine antagonises 5-HT_{2B} receptors, they do *not* modulate monoaminergic transmission (Millan et al., 2000b; Di Matteo et al., 2001). *Fourth*, agomelatine enhanced the spontaneous electrical activity of LC-localized adrenergic perikarya which are subject to tonic inhibition by 5-HT_{2C} receptors acting indirectly *via* (excitation of) GABAergic interneurones (Gobert et al., 2000; Millan et al., 2000b). Thus, as for other 5-HT_{2C} antagonists, agomelatine enhances ascending adrenergic transmission at least partially *via* actions at cell bodies. Excitation of GABAergic interneurones also intervenes in the inhibitory influence of 5-HT_{2C} receptors upon dopaminergic perikarya (Di Giovanni et al., 1999; Millan et al., 2000b) and, correspondingly, agomelatine abolished their inhibition by Ro60,0175. However, agomelatine neither increased the firing rate of dopaminergic neurones nor transformed their firing pattern into a bursting mode. By analogy, though SB206,533 facilitates electrical activity of VTA neurones, its influence is modest, while the selective 5-HT_{2C} antagonist, SB242,084, like agomelatine, does *not* excite VTA-dopaminergic perikarya (Di Giovanni et al., 1999; Millan et al., 2000b). Thus, tonic control of dopaminergic cell bodies is less pronounced than for their adrenergic counterparts and the principle locus of action of agomelatine in enhancing frontocortical dopaminergic transmission may be the FCX

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itself, probably at local GABAergic interneurons targetting dopaminergic terminals. An alternative explanation for the agomelatine-induced elevation in FCX levels of DA in the absence of changes in firing rate of VTA-dopaminergic neurones may be that it is secondary to the elevation in NA levels (Yamamoto and Novotney, 1998; Millan et al, 2000b). Thus, DA clearance in the FCX is partially effected by "NA transporters" on adrenergic terminals which can be saturated by high extracellular levels of NA.

Dopaminergic and adrenergic mechanisms in the FCX modulate cognitive-attentional performance, mood and motor behaviour (Arnsten, 1997; Millan et al., 2000b). These functions are profoundly perturbed in depressive states, in which a hypofrontality involving deficient catecholaminergic input to the FCX has been implicated (Willner, 1995; Millan et al., 2000b). Indeed, all clinically-effective antidepressant agents enhance extracellular levels of DA and NA in the FCX of freely-moving rats (Millan et al., 2000b). Thus, a reinforcement of FCX release of NA and DA by agomelatine may improve depressive states. Nevertheless, for agomelatine and other agents, it remains to be established whether an increase in FCX release of DA and NA is *sufficient* for therapeutic efficacy. Thus, caution should be exercised in relating this action of agomelatine to its effects in behavioural models of potential antidepressant properties in rodents (Bourin et al., 2002; Papp et al., 2003), and to its clinical actions in patients with major depression (Loo et al, 200).

Lack of influence of agomelatine upon subcortical DA release. While the significance of 5-HT_{2C} receptors in the control of mesolimbic as compared to nigrostriatal dopaminergic transmission remains controversial, there is a consensus that their tonic inhibition by 5-HT_{2C} receptors is *less* pronounced than for their frontocortical counterparts (Gobert et al., 2000; Millan et al., 2000b; De Deurwaerdère and Spampinato, 2001; Di Matteo et al., 2001). Indeed, mimicking SB206,553 and SB242,084 (Gobert et al., 2000), agomelatine did *not* modify dialysis levels of DA in the nucleus accumbens and striatum. Though behavioural studies suggest that melatonin interacts with dopaminergic mechanisms controlling motor behaviour in the nucleus accumbens and striatum, there is no evidence that it modulates DA release in these structures (Durlach-Misteli and Van Ree, 1992; Zisapel, 2002). Indeed, melatonin did not modify dialysis levels of DA in the nucleus accumbens and striatum.

Functional interactions between melatonin and serotonergic transmission. Melatonin indirectly interferes with activity at 5-HT_{2A} receptors (Dugovic et al., 1989; Eison et al., 1995), while 5-HT_{2A} sites modulate both the secretion of melatonin and its behavioural effects in rats (Gaffori and Van Ree, 1985; Govitrapong et al., 1991). Most pertinently, 5-HT_{2C} receptors are enriched in the suprachiasmatic nucleus (Sharma et al., 1997), a major locus of action of melatonin (Liu et al., 1997; Redman and Francis, 1998; Ying et al., 1998). Therein,

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5-HT_{2C} sites contributes to the influence of light upon neuronal rhythmicity and melatonin production (Kennaway and Moyer, 1998). Such interactions should be borne in mind in interpreting the functional profile of agomelatine.

Conclusions. In distinction to melatonin, agomelatine behaves as an antagonist at native, cerebral and cloned, human 5-HT_{2C} receptors in both cellular and *in vivo* paradigms. Correspondingly, blockade of 5-HT_{2C} receptors by agomelatine increases extracellular levels of DA and NA in FCX. It will be of interest to evaluate the contribution of the 5-HT_{2C} antagonist properties of agomelatine, and the accompanying enhancement of frontocortical dopaminergic and adrenergic transmission, to its influence upon mood.

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Table 1. Binding affinities of agomelatine as compared to melatonin at 5-HT_2 receptor subtypes

Drug	h5-HT_{2C}	h5-HT_{2B}	h5-HT_{2A}	r5-HT_{2A}	p5-HT_{2C}
Agomelatine	6.15 ± 0.04	6.59 ± 0.07	5.35 ± 0.08	<5.0	6.39 ± 0.02
Melatonin	<5.0	5.24 ± 0.09	<5.0	<5.0	<5.0

h = human, r = rat and p = porcine. Data are means ± SEMs of pK_i values derived from at least 3 independent determinations, each of which was performed in triplicate.

Table 2. Antagonist properties of agomelatine as compared to melatonin at h5-HT_{2C} and h5-HT_{2B} receptors.

	←——— h5-HT_{2C} ———→						←——— h5-HT_{2B} ———→
	Gq/11		Gi_3		[^{3}H]PI		[^{3}H]PI
Drug	pK_B	pA_2	pK_B	pA_2	pK_B	pA_2	pK_B
Agomelatine	6.02 ± 0.07	6.04	5.91 ± 0.04	5.99	6.06 ± 0.15	6.11	6.63 ± 0.08
Melatonin	<5.0	-	<5.0	-	<5.0	-	ND

Data are means ± SEMs derived from at least 3 independent determinations, each of which was performed in triplicate. ND = not determined.

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Figure 1.

Antagonist properties of agomelatine as compared to melatonin at cloned, human h5-HT_{2C} receptors as determined in a Scintillation Proximity Assay of the activation of Gq/11.

Panel A, Concentration-dependent blockade of the induction of [^{35}S]GTPγS binding by 5-HT (10 nM); Panel B, Displacement to the right of the concentration-response curve for 5-HT in the presence of incremental concentrations of agomelatine and Panel C, Schild transformation of the data of Panel B. The curves shown are from representative experiments, each of which was performed in triplicate. For each concentration, values shown are the means ± SEMs of the triplicates.

Figure 2.

Antagonist properties of agomelatine as compared to melatonin at cloned, human h5-HT_{2C} receptors as determined in a Scintillation Proximity Assay of the activation of Gi_3.

Panel A, Concentration-dependent blockade of the induction of [^{35}S]GTPγS binding by 5-HT (100 nM); Panel B, Displacement to the right of the concentration-response curve for 5-HT in the presence of incremental concentrations of agomelatine and Panel C, Schild transformation of the data of Panel B. The curves shown are from representative experiments, each of which was performed in triplicate. For each concentration, values shown are the means ± SEMs of the triplicates.

Figure 3.

Antagonist properties of agomelatine as compared to melatonin at cloned, human h5-HT_{2C} receptors as determined in a [^{3}H]PI depletion assay of the activation of Phospholipase C.

Panel A, Concentration-dependent blockade of 5-HT (10 nM)-induced [^{3}H]PI depletion; Panel B, Displacement to the right of the concentration-response curve for 5-HT in the presence of incremental concentrations of agomelatine and Panel C, Schild transformation of the data of Panel B. The curves shown are from representative experiments, each of which was

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performed in triplicate. For each concentration, values shown are the means ± SEMs of the triplicates.

Figure 4.

Antagonist properties of agomelatine as compared to melatonin at cloned, human h5-HT_{2B} receptors as determined in a [^{3}H]PI depletion assay of the activation of Phospholipase C.

Concentration-dependent blockade of 5-HT (30 nM)-induced [^{3}H]PI depletion. The curves shown are from representative experiments, each of which was performed in triplicate. For each concentration, values shown are the means ± SEMs of the triplicates.

Figure 5.

Antagonist properties of agomelatine as compared to melatonin at 5-HT_{2C} receptors *in vivo* as determined by blockade of the induction of penile erections by the 5-HT_{2C} agonists, Ro60,0175 and Ro60,0332.

Panel A, Induction of penile erections by Ro60,0175 (0.16 - 2.5 mg/kg, s.c.) and Ro60,0332 (0.16 - 10.0 mg/kg, s.c.) as compared to agomelatine (0.63 and 40.0 mg/kg, i.p.) and melatonin (10.0 mg/kg, i.p.); Panel B, Blockade of the action of Ro60,0175 (1.25 mg/kg, s.c.) by agomelatine (2.5 - 80.0 mg/kg, i.p.) and Panel C, Blockade of the action of Ro60,0332 (2.5 mg/kg, s.c.) by agomelatine (2.5 - 40.0 mg/kg, i.p.). Closed symbols are for drug treatment and open symbols for vehicle (VEH) controls. Data are means ± SEMs. N ≥ 8 per value. Asterisks indicate significance of differences to respective vehicle values in Dunnett's test. * P < 0.05.

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Figure 6.

Influence of agomelatine upon extracellular levels of NA and DA in the frontal cortex (FCX) as compared to extracellular levels of DA in the nucleus accumbens (ACC) and striatum (STR) of freely-moving rats.

Data are means ± SEMs of DA and NA levels expressed relative to basal, pre-treatment values (defined as 100 %). These were, 1.1 ± 0.1, 8.3 ± 1.2 and 13.0 ± 1.5 pg/20µl dialysate for DA in the FCX, nucleus accumbens and striatum, respectively, and 1.49 ± 0.05 pg/20 µl dialysate for NE in the FCX. N ≥ 5 per value. All drug doses are in mg/kg, i.p. Asterisks indicate significance of drug *versus* vehicle values in Dunnett's test. * $P < 0.05$.

Figure 7.

Lack of influence of the selective melatonin antagonist, S22153, upon the increase in extracellular levels of DA and NA elicited by agomelatine in the frontal cortex of freely-moving rats.

Data are means ± SEMs. N ≥ 5 per value. All drug doses are in mg/kg, i.p.

Figure 8.

Influence of agomelatine as compared to melatonin upon the electrical activity of adrenergic perikarya in the locus coeruleus.

Panel A, Dose-dependent influence of agomelatine upon firing rate, Panel B, representative spike from a dopaminergic neurone and Panel C, Influence of agomelatine followed by the α_2-adrenoceptor agonist, clonidine, upon the electrical discharge of a representative neurone. For the upper panel, data are means ± SEMs. N ≥ 5 per value. Closed symbols are for drug treatment and open symbols for vehicle (VEH) controls. All drug doses are in mg/kg, i.v. Asterisks indicate significance of differences to respective vehicle values in Newman-Keuls test (paired data). * $P < 0.05$. For melatonin (Student's two-tailed t-test), $P > 0.05$.

Figure 9.

Influence of agomelatine as compared to melatonin upon the electrical activity of dopaminergic perikarya in the ventral tegmental area, and blockade by agomelatine of the action of Ro60,0175.

Panel A, Lack of influence of agomelatine and melatonin upon basal firing rate as compared to Ro60,0175 and reversal of the inhibitory influence of Ro60,0175 by agomelatine as compared to melatonin; Panel B, Representative spike from an adrenergic neurone and Panel C: Representative neurone showing the blockade by agomelatine of the action of Ro60,0175 followed by inhibition of firing with the dopaminergic agonist, apomorphine. For Panel A, data are means ± SEMs. $N \geq 5$ per value. All drug doses are in mg/kg, i.v. Asterisks indicate significance of differences in Dunnett's test. * $P < 0.05$

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FIG. 1

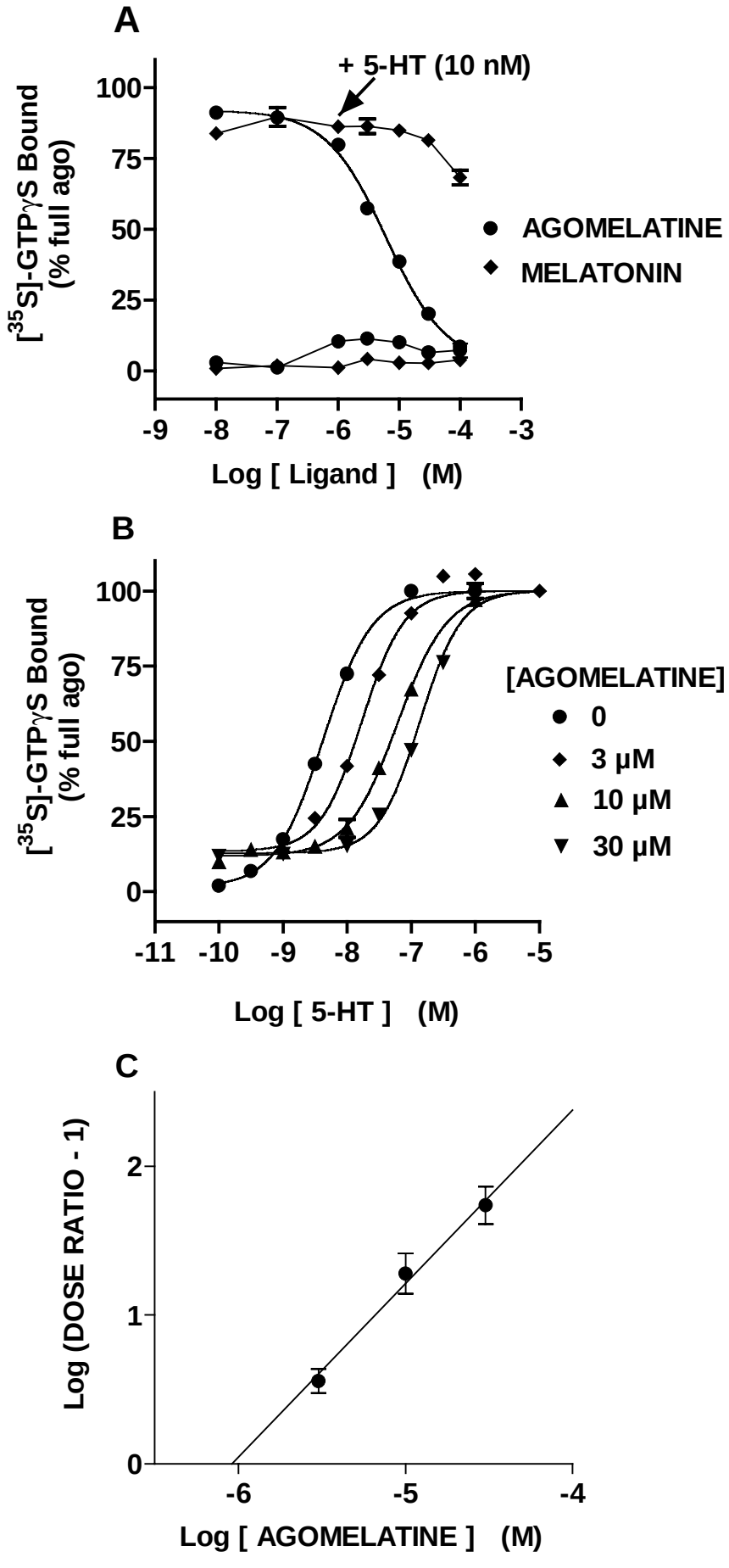

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FIG. 2

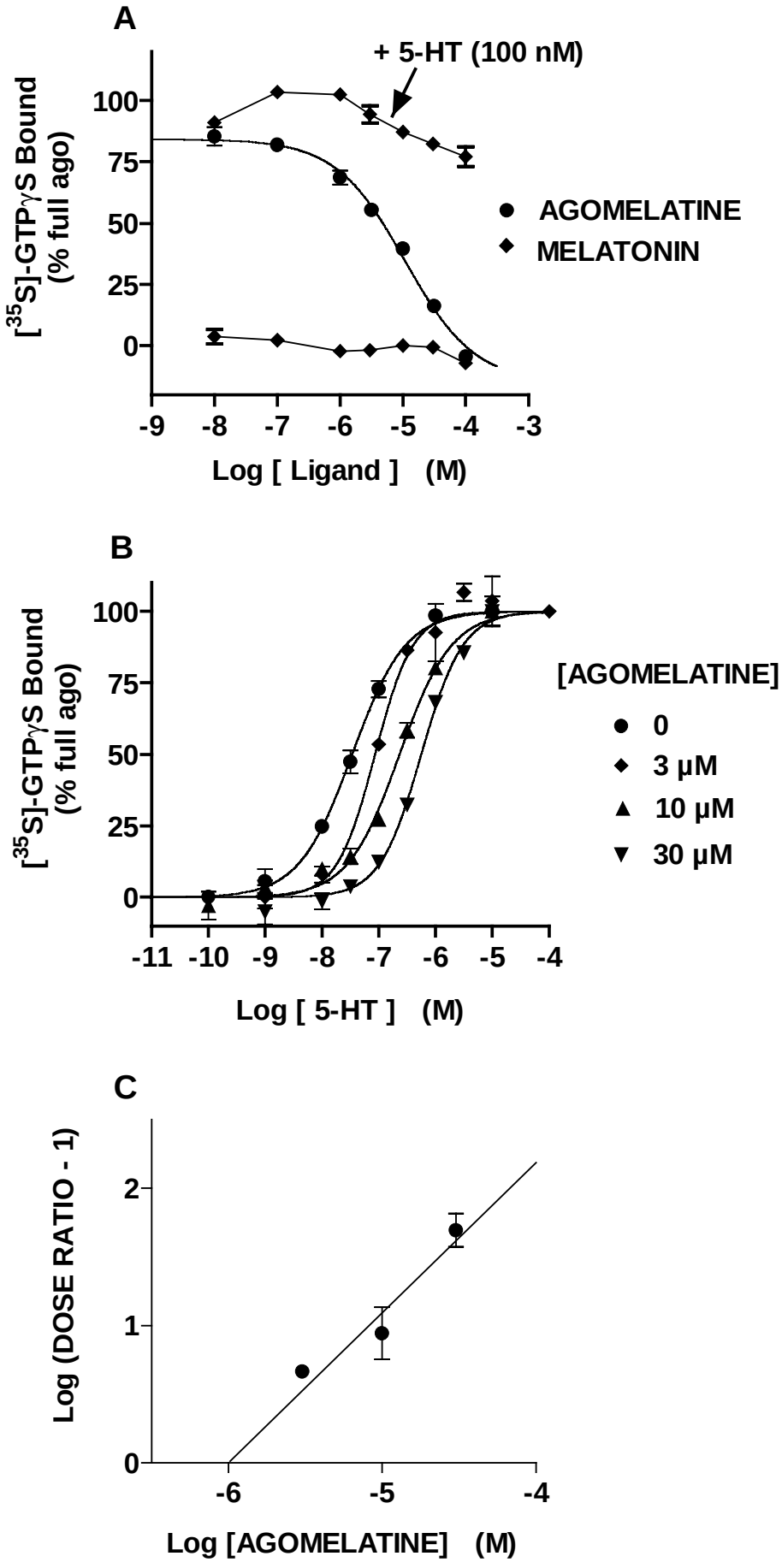

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FIG. 3

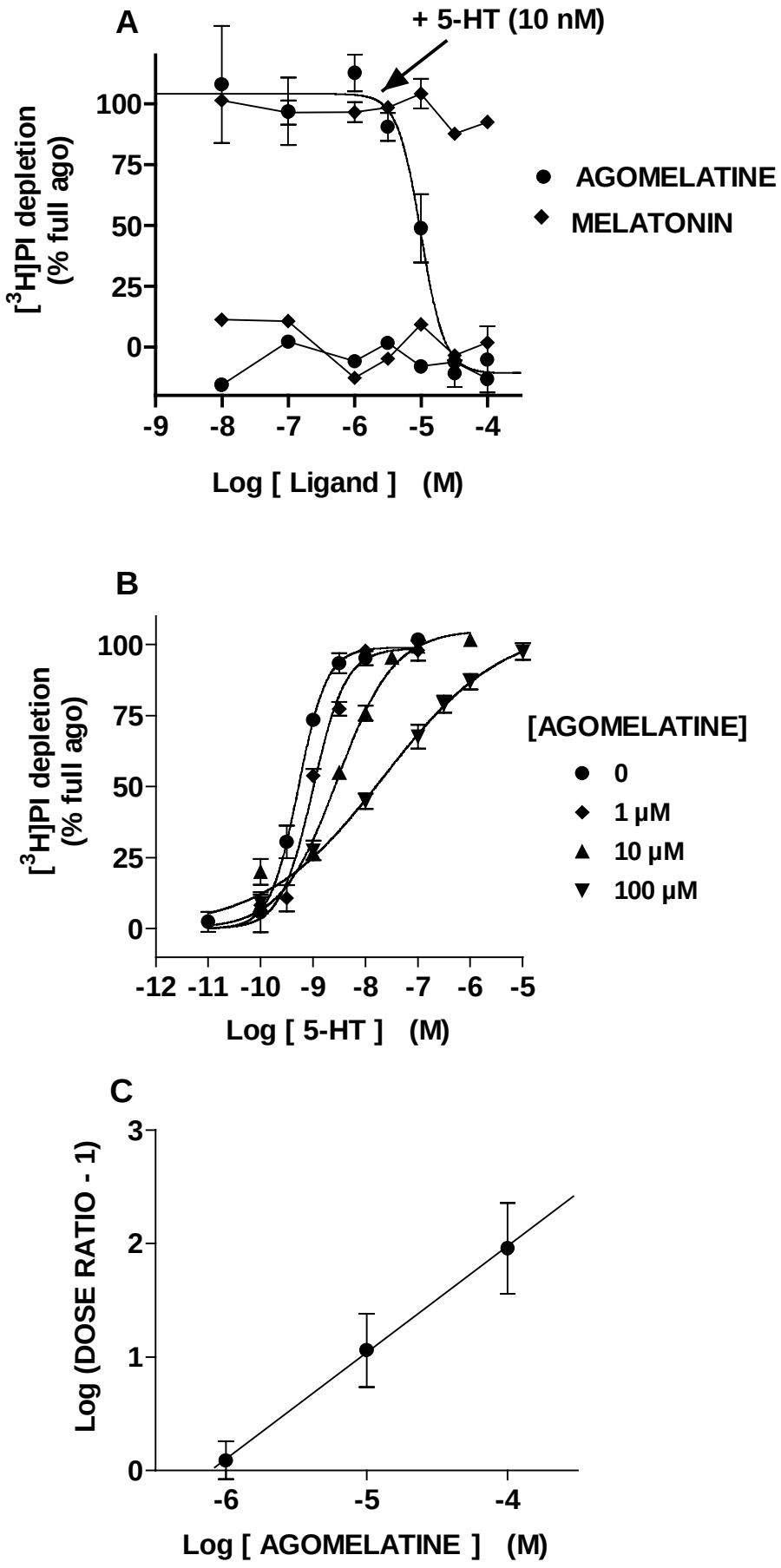

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FIG. 4

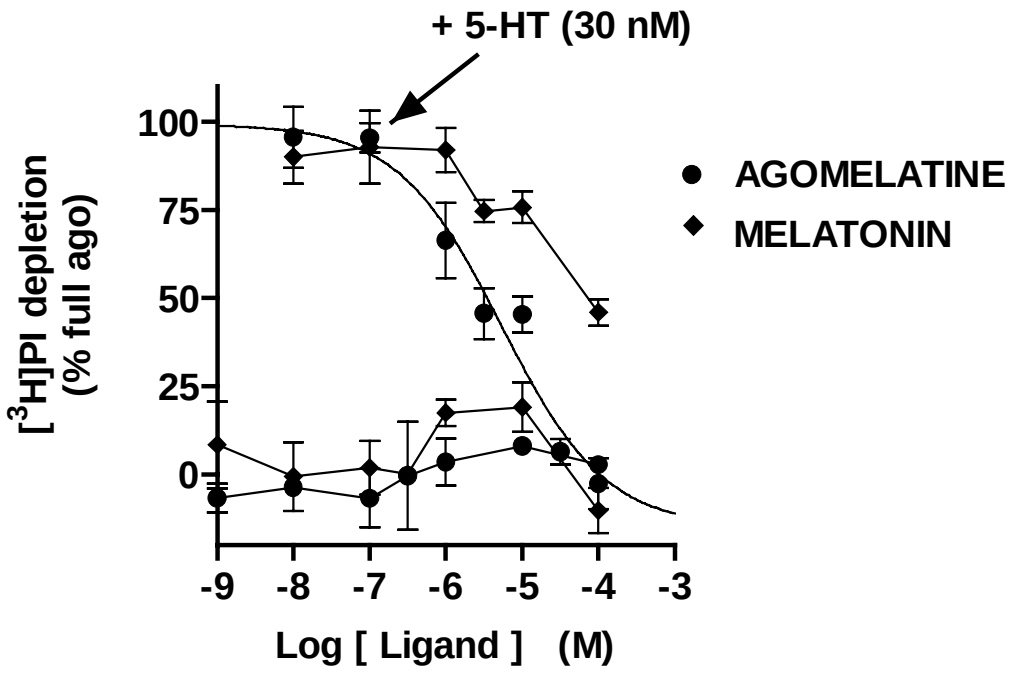

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FIG. 5

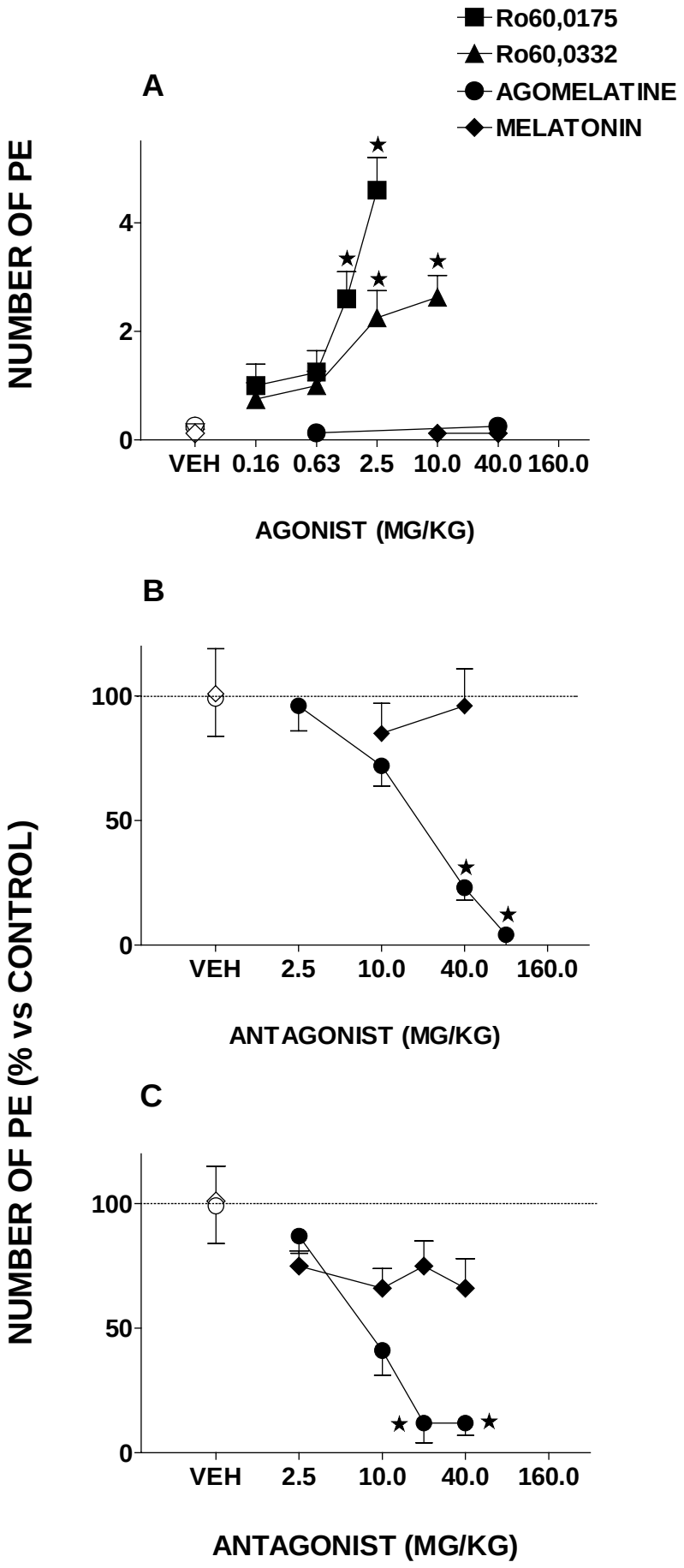

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FIG. 6

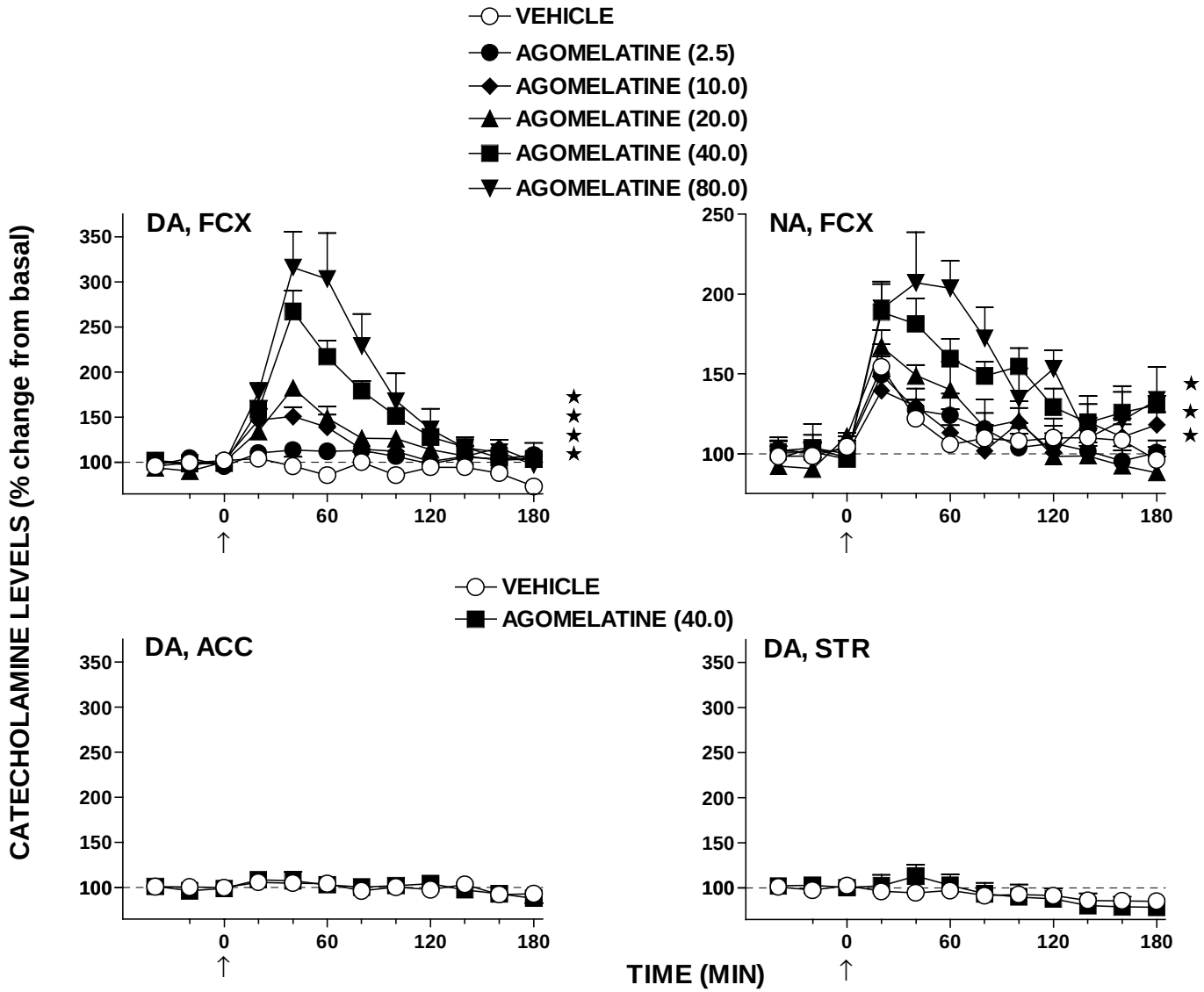

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FIG. 7

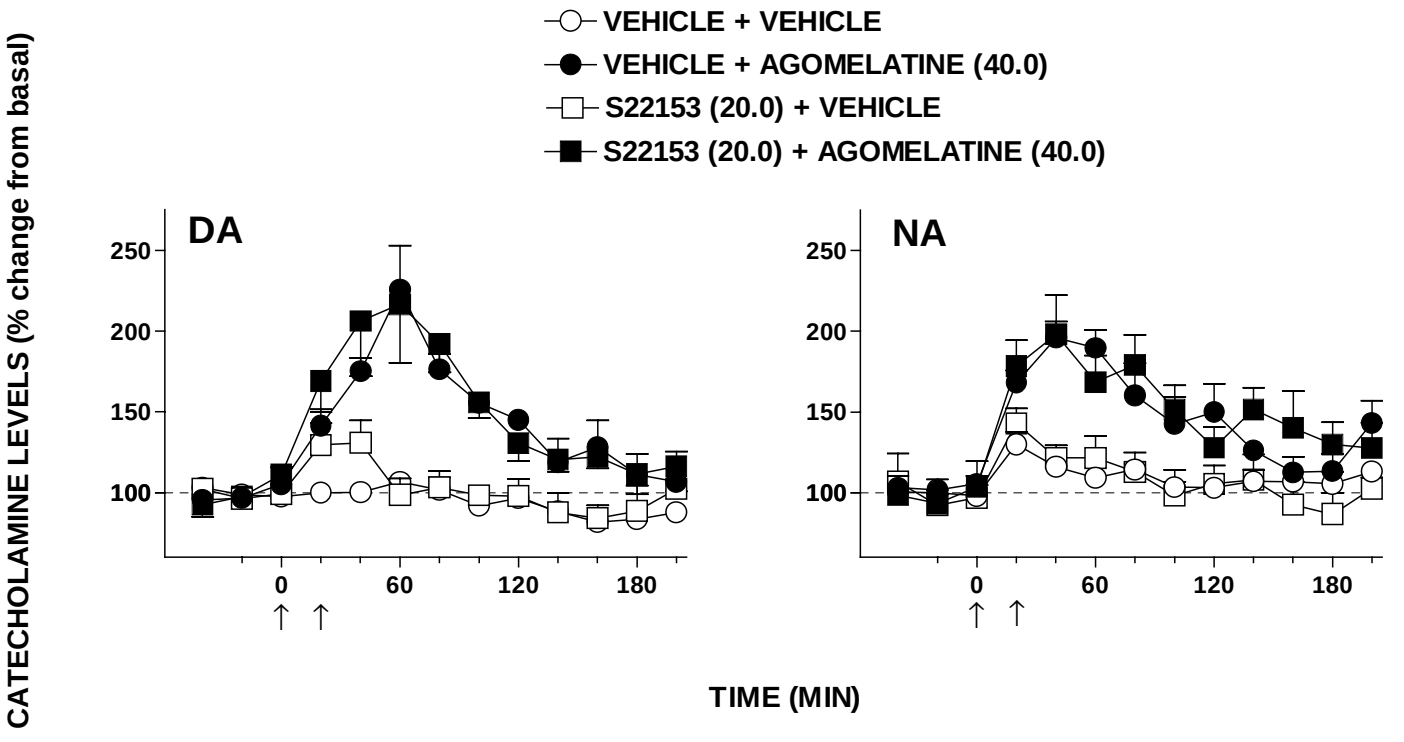

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FIG. 8

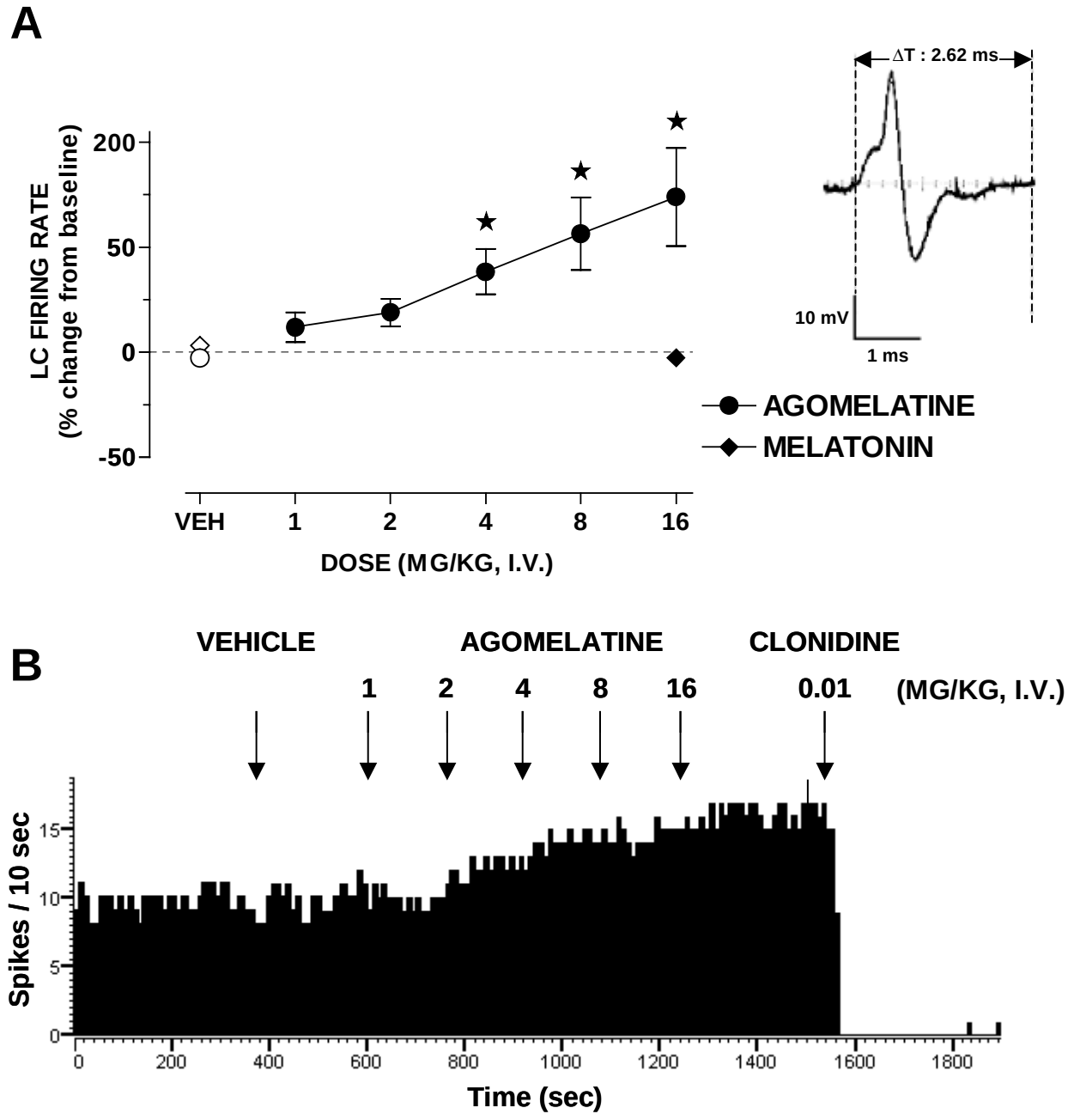

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FIG. 9