Psychopharmacology (2005) 177: 448–458
DOI 10.1007/s00213-004-1962-z

ORIGINAL INVESTIGATION

Mark J. Millan · Mauricette Brocco · Alain Gobert · Anne Dekeyne

Anxiolytic properties of agomelatine, an antidepressant with melatoninergic and serotonergic properties: role of 5-HT_{2C} receptor blockade

Received: 6 April 2004 / Accepted: 9 June 2004 / Published online: 31 July 2004

Abstract *Rationale:* The novel antidepressant agent, agomelatine, behaves as an agonist at melatonin receptors and as an antagonist at serotonin $(5\text{-HT})_{2C}$ receptors. *Objectives:* To determine whether, by virtue of its antagonist properties at 5-HT_{2C} receptors, agomelatine elicits anxiolytic properties in rats. *Methods:* Employing a combined neurochemical and behavioural approach, actions of agomelatine were compared to those of melatonin, the selective 5-HT_{2C} receptor antagonist, SB243,213, and the benzodiazepine, clorazepate. *Results:* In unfamiliar pairs of rats exposed to a novel environment, agomelatine enhanced the time devoted to active social interaction, an action mimicked by clorazepate and by SB243,213. In a Vogel conflict procedure, agomelatine likewise displayed dose-dependent anxiolytic activity with a maximal effect comparable to clorazepate, and SB243,213 was similarly active in this procedure. In a plus-maze procedure in which clorazepate significantly enhanced percentage entries into open arms, agomelatine revealed only modest activity and SB243,213 was inactive. Further, like SB243,213, and in contrast to clorazepate, agomelatine did not suppress ultrasonic vocalizations emitted by rats re-exposed to an environment associated with an aversive stimulus. Whereas clorazepate reduced dialysate levels of 5-HT and noradrenaline in hippocampus and frontal cortex of freely moving rats, agomelatine did not affect extracellular levels of 5-HT and elevated those of noradrenaline. SB243,213 acted similarly to agomelatine. Melatonin, which did not modify extracellular levels of 5-HT or noradrenaline, was ineffective in all models of anxiolytic activity. Furthermore, the selective melatonin antagonist, S22153, did not modify anxiolytic properties of agomelatine in either the social interaction or the Vogel Conflict tests. *Conclusions:* In contrast to melatonin, and reflecting blockade of 5-HT_{2C} receptors, agomelatine is active in several models of anxiolytic properties in rodents. The anxiolytic profile of agomelatine differs from that of benzodiazepines from which it may also be distinguished by its contrasting influence on corticolimbic monoaminergic pathways.

Keywords Melatonin · 5-HT_{2C} receptors · Anxiety · Anxiolytic · Conflict · Frontal cortex · Dorsal hippocampus

M. J. Millan (✉) · M. Brocco · A. Gobert · A. Dekeyne
Department of Psychopharmacology, Centre de Recherches de Croissy, Institut de Recherches Servier,
125 Chemin de Ronde, Croissy/Seine,
78290 Paris, France
e-mail: mark.millan@fr.netgrs.com
Tel.: +33-1-55722425
Fax: +33-1-55722470

Introduction

The novel, clinically active antidepressant agent, agomelatine (Valdoxan, S20098) (Lôo et al. 2002b; Papp et al. 2003), behaves as a potent agonist at melatonin MT_1 and MT_2 sites (Yous et al. 1992; Ying et al. 1996; Van Reeth et al. 2001). It also acts as a—less potent—antagonist at serotonin (5-HT_{2C}) receptors both in vitro and in vivo (Chagraoui et al. 2003; Millan et al. 2003). Blockade of 5-HT_{2C} receptors, which are concentrated in the frontal cortex (FCX), hippocampus and amygdala (Clemett et al. 2000; Lopez-Gimenez et al. 2002; Millan 2003), has been implicated in the antidepressant profile of agomelatine (Millan et al. 2003). Antagonist actions at 5-HT_{2C} receptors may also be associated with anxiolytic properties (Deakin 1994; Griebel et al. 1997; Millan 2003).

Thus, mice genetically lacking 5-HT_{2C} receptors display reduced anxiety (Das and Tecott 1996) while, in several experimental models, 5-HT_{2C} receptor agonists and antagonists display anxiogenic and anxiolytic properties, respectively (Cervo and Samanin 1995; Kennett et al. 1996, 1997; Griebel et al. 1997; Dekeyne et al. 2000a; Millan et al. 2001; Millan 2003). As concerns antidepressants, the "atypical" agents, mianserin and mirtazapine, which behave as potent antagonists at 5-HT_{2C} receptors, display anxiolytic properties in animals and in humans (Rocha et al. 1994; Casacalenda and Boulenger 1998;

Millan 2003). Further, while acute administration of selective 5-HT reuptake inhibitors elicits anxiety in rodents via the indirect activation of 5-HT_{2C} receptors, their chronic administration reduces anxiety in parallel with a down-regulation of 5-HT_{2C} receptors (Bristow et al. 2000; Dekeyne et al. 2000b). This observation mirrors their clinical utilization in the long-term treatment of anxious states (see Millan 2003).

Potential anxiolytic properties of agomelatine are also of interest in light of certain observations that engagement of melatoninergic receptors is involved in the response to anxious states. First, melatonin secretion is under the facilitatory control of pineal β-adrenoceptors (ARs) which are innervated by stress-sensitive, sympathetic adrenergic neurones (Borjigin et al. 1999). Accordingly, a diversity of stressful and anxiogenic stimuli enhance pineal output of melatonin (Golombek et al. 1996; Millan 2003). Second, clinical studies suggest that treatment with melatonin reduces pre-operative anxiety (Naguib and Samarkandi 2000). Third, there are reports that melatonin expresses anxiolytic properties in rodents (Niles 1991; Guardiola-Lemaitre et al. 1992; Pierrefiche et al. 1993; Golombek et al. 1996; Kopp et al. 1999a,b, 2000; Nava and Carta 2001). It has been proposed that its actions are, at least partially, expressed via γ-amino butyric acid (GABA)-related mechanisms: this has, however, been disputed (preceding citations). Notwithstanding these observations, several findings argue against a major or generalized role of melatoninergic receptors in the control of anxious states. Thus, as compared to the robust and broad-based anxiolytic actions of benzodiazepines (BZPs), with few exceptions (Golombek et al. 1993; Naranjo-Rodriguez et al. 2000; see Millan 2003), anxiolytic actions of melatonin are only exerted in models of exploratory behaviour, and only at specific phases of the light/dark cycle (Golombek et al. 1993; Pierrefiche et al. 1993; Kopp et al. 1999a, 2000). Furthermore, though BZPs suppress the nocturnal secretion of melatonin by a reduction in the activity of the rate-limiting enzyme for its synthesis, *N*-acetyltransferase (Krueger 1991; Atsmon et al. 1996), this action is related to their hypnotic and sedative actions rather than their anxiolytic properties (Borjigin et al. 1999). Finally, melatonin reduces BZP requirements in humans for induction of sleep, but not for expression of their anxiolytic properties (Cardinali et al. 2002).

The above observations suggest that the 5-HT_{2C} antagonist actions of agomelatine (rather than its stimulation of $MLT_{1/2}$ receptors) are more likely to be associated with anxiolytic properties. To evaluate this hypothesis, we examined potential anxiolytic actions of agomelatine in two rodent models consistently responsive to selective 5-HT_{2C} receptor antagonists: the social interaction and Vogel conflict tests (File and Seth 2003; Millan and Brocco 2003). In addition, we employed two procedures in which such agents have generally proven inactive: the plus-maze test and ultrasonic vocalization (USV) tests (Molewijk et al. 1995; Wall and Messier 2001; Sánchez 2003). To confirm the potential role of 5-HT_{2C} sites in the actions of agomelatine, comparative studies were undertaken with melatonin and with the selective 5-HT_{2C} receptor antagonist, SB243,213 (Wood et al. 2001). Further, we examined the sensitivity of the anxiolytic actions of agomelatine to blockade by the selective melatonin (MT_1/MT_2) receptor antagonist, S22153 (Kopp et al. 1999b; Weibel et al. 1999). In parallel we evaluated anxiolytic actions of the prototypical BZP, clorazepate. Finally, inasmuch as a suppression of corticolimbic serotonergic and adrenergic transmission is implicated in the anxiolytic properties of BZPs (Gorman et al. 2000; Millan 2003), we determined the influence of agomelatine as compared to the other agents on extracellular levels of 5-HT and noradrenaline (NA) in the dorsal hippocampus and FCX of freely moving rats.

Materials and methods

Animals Unless otherwise specified, these studies employed male Wistar rats of 200–250 g and NMRI mice of 22–25 g (Iffa Credo, l'Arbresle, France) housed in sawdust-lined cages with unrestricted access to standard chow and water. There was a 12 h/12 h light/dark cycle with lights on at 7.30 a.m. Laboratory temperature and humidity were 21±0.5°C and 60±5%, respectively. Animals were adapted to laboratory conditions for at least a week prior to testing and all experiments were performed during the light period of the cycle. All animal use procedures conformed to international European ethical standards (86/609-EEC) and the French National Committee (décret 87/848) for the care and use of laboratory animals.

Social interaction test As described in detail previously (Millan et al. 2001), male Sprague-Dawley rats of 240–260 g (Charles River, Saint-Aubin-les-Elbeuf, France) were individually housed for 5 days before testing. On the test day, they were placed in weight-matched pairs (±5 g) in opposite corners of a highly illuminated (300 lux), open-topped arena (57×36×30 cm^3) for a 10-min session. Rats of the same pair received the same drug treatment. Analysed data were the duration of "active" contact between the animals; i.e. the time spent in grooming, following, sniffing, biting, jumping or crawling over or under the other animal. If animals remained adjacent to each other without any movement for more than 10 s, scoring was discontinued until active social interaction resumed.

Vogel conflict test As previously described (Millan et al. 2001), the test was conducted in polycarbonate cages (32×25×30 cm^3) with a grid floor and with the spout of a water bottle located 6 cm above the floor. Both the grid and the spout were connected to an Anxiometer (Columbus Instruments, Ohio, USA) used to record licks and deliver electrical shocks. During the 3 days preceding testing, rats were housed by four and were restricted to 1 h per day access to tap water (from 9:00 to 10:00 a.m.). On day 4, just after water delivery, they were isolated in cages with a grid-floor. Testing took place on day 5. Rats were individually placed in a test cage and the test session was initiated after the rat had made 20 licks of the spout and received a single shock (300 μA and 0.5 s) via the spout. Over 3 min, a shock was then delivered every 20 licks. Analysed data were the number of licks during the test session. In one group of vehicle-treated animals, no shock was delivered, in order to provide a baseline. The percentage drug effect was computed as [(drug−vehicle)/(vehicle non-shocked−vehicle)].

Plus-maze test As previously described (Millan et al. 2001), the experiments were performed in a white-mat-painted, plus-maze constructed of wood and elevated to a height of 50 cm. The apparatus comprised two open arms (50×10 cm^2) and two enclosed arms of the same dimensions, with walls 40 cm high. The two open

arms were opposite to each other. On the test day, each rat was placed in the central square of the maze facing one of the enclosed arms for a 5 min session. The number of entries and time spent in open and enclosed arms were recorded. Analysed data were the total number of entries, the number of entries in enclosed arms, the percentage entries in open arms and the percentage time spent in open arms.

Ultrasonic vocalization test As previously described (Millan et al. 2001), the rats were initially placed in a chamber equipped with a grid-floor and were exposed to six randomly distributed electric shocks (800 μA and 8 s) over a 7-min period. Twenty-four hours later, they were placed in the chamber for 2 min and received a single shock. They were returned to the chamber 30 min later and USVs recorded for 10 min. Rats emitting USVs less than 90 s in duration were not examined further. Twenty-four hours later, the procedure of the day before was replicated with drugs or vehicle administered immediately after the 2-min session. Data analysed were the total duration of USVs recorded over the 10-min session.

Determination of dialysate levels of monoamines The protocol employed is described in detail elsewhere (Gobert et al. 2000). Briefly, the influence of drugs on levels of 5-HT and NA in single dialysate samples of the dorsal hippocampus and FCX was determined employing high performance liquid chromatography plus coulometric detection in freely moving rats implanted 1 week prior to study with a guide cannula at the following coordinates. Dorsal hippocampus: AP=−3.6 from bregma, *L*=±1.2 and *H*=−2.3 from dura and FCX, AP=+2.2 from bregma, *L*=±0.6 and *H*=−0.2 from dura. Samples were taken every 20 min. Basal monoamine levels were monitored over 1 h, then drugs injected, and samples taken for a further 140 min. Changes were expressed relative to basal values (defined as 100%). The detection limits for 5-HT and NA were, in each case, 0.1–0.2 pg/sample.

Spontaneous locomotion in rats As previously described (Millan et al. 2001), rats were individually placed for 12 min in transparent polycarbonate cages (45×30×20 cm^3) equipped with two rows of photocells 4 cm above the floor and 24 cm apart. Analysed data were locomotion counts over the 12 min session, with a count corresponding to the consecutive interruption of two infrared beams.

Drugs All drugs were suspended in a few drops of Tween 80 and administered IP In models of potential anxiolytic properties, and of the influence of drugs on motor behaviour, animals were administered with drug or vehicle 30 min before testing and full dose–response curves were undertaken for all drugs. In the antagonism studies performed in the social interaction and Vogel conflict tests, S22153 or vehicle was administered 15 min before agomelatine or vehicle. Melatonin and clorazepate dipotassium were obtained from Sigma (Saint Quentin-Fallavier, France). Agomelatine, SB243,213 (5-methyl1-1[(2-[(2-methyl-3-pyridyl)oxyl]-5-pyridil] carbamoyl]-6-trifluoromethylindoline) hydrochloride and S22153 (*N*-[2-(5-ethyl-benzo[b] thien-3-yl)ethyl] acetamide), were synthesized by Servier chemists.

Statistics In all behavioural studies, dose-effects were analysed employing one-way analyses of variance (ANOVA) followed by Dunnett's test. Where computable (USV and motor procedures), Inhibitory $Dose_{50}$s (ID_{50}s) plus 95% confidence limits (CL) were calculated. In the dialysis studies, ANOVA with dose as between factor was performed over 20–140 min.

Results

Social interaction test (Fig. 1, Table 1) At doses of 2.5 mg/kg and 10.0 mg/kg, agomelatine elicited a significant increase in the time devoted to active social interaction in pairs of unfamiliar rats introduced into a novel environment (Fig. 1). A further increase in dose resulted in an inflection of the dose–response curve. Though melatonin tended to increase the time of social interaction at a dose of 40.0 mg/kg, this effect did not attain statistical significance (Fig. 1). Further, at the highest dose tested, melatonin resulted in a significant decrease in active social interaction. Clorazepate induced a significant elevation in active social interaction at a dose of 10.0 mg/kg (Fig. 1). SB243,213 significantly increased social interaction at doses of 0.63 mg/kg and 2.5 mg/kg (Fig. 1). For both clorazepate and SB243,213, a further increase in doses resulted in a loss of effect. ANOVAs were as follows: agomelatine, $F(5,28)=5.3$, $P<0.01$; melatonin, $F(6,46)=5.9$, $P<0.001$; clorazepate, $F(3,20)=5.3$, $P<0.01$ and SB243,213, $F(5,32)=3.3$, $P<0.05$.

Vogel conflict test (Fig. 2, Table 1) Agomelatine (2.5–80.0 mg/kg) elicited a dose-dependent increase in punished responses in the Vogel conflict test (Fig. 2). In contrast, over a similar dose-range, melatonin was inactive. Clorazepate (5.0–20.0) displayed dose-dependent activity with a maximal effect similar to that of agomelatine. SB243,213 (10–40.0 mg/kg) also dose-dependently increased punished responses in this procedure. ANOVA were as follows: agomelatine, $F(4,46)=6.2$, $P<0.001$; melatonin, $F(4,47)=1.5$, $P>0.05$; clorazepate, $F(4,56)=3.2$, $P<0.05$ and SB243,213, $F(3,34)=6.1$, $P<0.01$.

Lack of influence of the selective melatonin antagonist, S22153, on the actions of agomelatine (Fig. 3) The selective melatonin antagonist, S22153, was used at a dose (20.0 mg/kg) previously demonstrated to abolish the

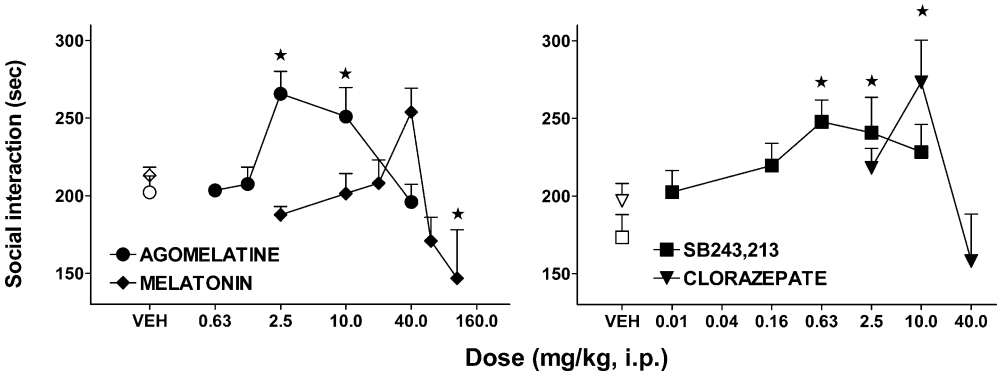

Fig. 1 Actions of agomelatine, melatonin, clorazepate and SB243,213 in the social interaction procedure in rats. *VEH* vehicle. Data are means±SEMs. *n*=5 per value. For ANOVA, see Results. *Asterisks* indicate significance of differences to respective vehicle values in Dunnett's test. *$P<0.05$

Table 1 Summary of overall functional profiles of drugs on acute administration in models predictive of anxiolytic properties as compared to their influence on extracellular level of 5-HT and NA. *Yes* active and *No* inactive; *HIPP* hippocampus; *FCX* frontal cortex; *USV* ultrasonic vocalizations; *PM* plus-maze; *SI* social interaction; *5-HT* serotonin; *NA* noradrenaline and *SSRI* selective 5-HT reuptake inhibitor; – no effect; ↓ or ↑ decrease or increase, respectively

Drug	Class	Vogel	USV	PM	SI	HIPP/FCX	
						5-HT	NA
Agomelatine	Melatonin agonist/5-HT_{2C} antagonist	Yes	No	Yes[a]	Yes	–	↑[b]
Melatonin	Melatonin agonist	No	No	No	No	–	–[b]
Clorazepate	Benzodiazepine	Yes	Yes	Yes	Yes	↓	↓
Chlordiazepoxide	Benzodiazepine	Yes	Yes	Yes	Yes	↓	↓
Diazepam	Benzodiazepine	Yes	Yes	Yes	Yes	↓	↓
SB243,213	5-HT_{2C} antagonist	Yes	No	No	Yes	–	↑
SB242,084	5-HT_{2C} antagonist	Yes	No	No	Yes	–	↑
SB206,553	5-HT_{2C} antagonist	Yes	No	No	Yes	–	↑
Citalopram	SSRI	No	No	No	No[c]	↑	–

[a]A significant effect of agomelatine was observed only on % entries into open arms of the plus-maze procedure
[b]See also Millan et al. 2003
[c]Anxiogenic. In addition to drugs examined herein, to facilitate comparisons, data are summarized for several other agents previously studied in these procedures under identical conditions, including the selective serotonin reuptake inhibitor (SSRI), citalopram (Dekeyne et al. 2000a; Gobert et al. 2000; Millan et al. 2000a, 2001)

influence of melatonin on circadian rhythms (Weibel et al. 1999). In the social interaction procedure, S22153 was inactive alone and failed to modify the action of agomelatine (2.5 mg/kg). Two-way ANOVA was as follows: influence of agomelatine, $F(1,20)=18.9$, $P<0.001$; influence of S22153, $F(1,20)=1.1$, $P>0.05$ and interaction, $F(1,20)=0.3$, $P>0.05$. In the Vogel conflict test, S22153 (20.0 mg/kg), which was ineffective alone, did not modify the action of agomelatine (80.0 mg/kg). Two-way ANOVA was as follows: influence of agomelatine, $F(1,62)=43.2$, $P<0.001$; influence of S22153, $F(1,62)=1.1$, $P>0.05$ and interaction, $F(1,62)=1.1$, $P>0.05$.

Plus-maze test (Fig. 4, Table 1) Agomelatine evoked a dose-dependent increase in the percentage of entries into open arms of the plus-maze, with a slight inflection of the dose–response curve at the highest dose tested (80.0 mg/kg). Statistical significance was obtained at doses of 40.0 mg/kg and 80.0 mg/kg. Correspondingly, a significant decrease of entries in enclosed arms was observed at a dose of 80.0 mg/kg. Biphasic dose–response curves were obtained for percentage of time spent in open arms and for total entries, though no dose of agomelatine actually resulted in a statistically significant change. Melatonin (10.0–120.0 mg/kg) did not elicit a statistically significant change in percentage open arm time or entries, or in enclosed arms or total entries. Clorazepate (0.63–40.0 mg/kg) evoked a dose-dependent and significant elevation in percentage entries and time in open arms, in number of entries into enclosed arms, and in total entries. However, for total entries, its dose–response curve inflected at the highest dose evaluated. Over a broad dose-range (0.16–40.0 mg/kg), SB243,213 had no significant effect on either time or percentage entries in open arms, though it slightly and non-significantly decreased both enclosed arms and total entries at the highest dose. ANOVA were as follows: for % entries in open arms, agomelatine, $F(4,44)=5.5$, $P<0.01$; melatonin, $F(4,41)=1.0$, $P>0.05$; clorazepate, $F(4,42)=9.1$, $P<0.001$ and SB243,213, $F(5,42)=0.5$, $P>0.05$. For % time in open arms, agomelatine, $F(4,44)=2.2$, $P>0.05$; melatonin, $F(4,41)=2.2$, $P>0.05$; clorazepate, $F(4,42)=15.8$, $P<0.001$ and SB243,213, $F(5,42)=0.4$, $P>0.05$. For entries in enclosed arms, agomelatine, $F(4,44)=2.8$, $P<0.05$; melatonin, $F(4,41)=0.3$, $P>0.05$; clorazepate, $F(4,42)=4.7$, $P<0.01$ and SB243,213, $F(5,42)=1.0$, $P>0.05$. For total entries, agomelatine, $F(4,44)=1.9$, $P>0.05$; melatonin, $F(4,41)=0.8$, $P>0.05$; clorazepate, $F(4,42)=9.5$, $P<0.001$ and SB243,213, $F(5,42)=0.7$,

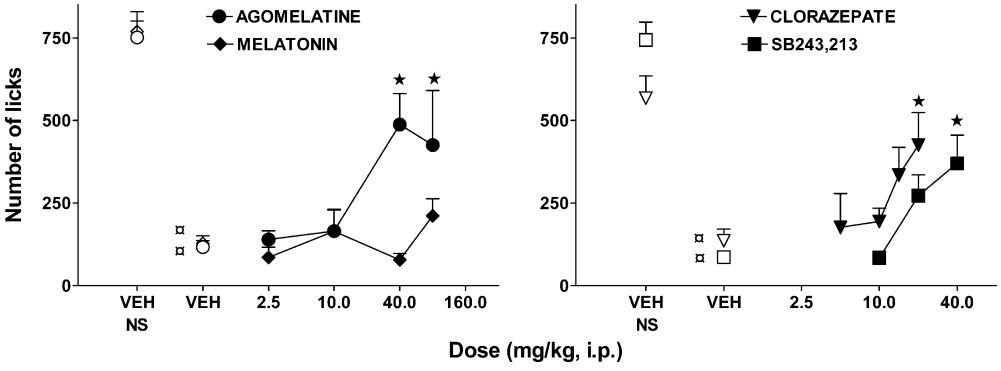

Fig. 2 Actions of agomelatine, melatonin, clorazepate and SB243,213 in the Vogel Conflict test in rats. *VEH* vehicle. Data are means±SEMs. n=6–19 per value. For ANOVA, see Results. The *open asterisks* indicate the significance of differences to vehicle non-punished value, in Student's *t*-test. The *closed asterisks* indicate the significance of differences to vehicle values in Dunnett's test. *$P<0.05$

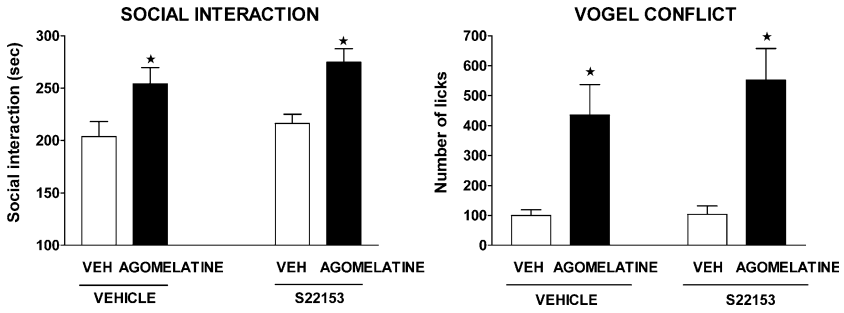

Fig. 3 Lack of influence of the melatonin receptor antagonist, S22153, on the anxiolytic properties of agomelatine in the social interaction test and the Vogel conflict test in rats. Data are means ±SEMs. *VEH* vehicle. Social interaction procedure: doses were 2.5 mg/kg and 20.0 mg/kg, IP for agomelatine and S22153, respectively, n=6 per value. Vogel conflict procedure: doses were 80.0 mg/kg and 20.0 mg/kg, IP for agomelatine and S22153, respectively, n=11 per value. For ANOVA, see Results. *Asterisks* indicate significance of differences of vehicle/agomelatine to vehicle/vehicle and of S22153/agomelatine to S22153/vehicle values in Dunnett's test. *P<0.05

P>0.05.

Ultrasonic vocalization test (Fig. 5, Table 1) Administered over a dose-range equivalent to that evaluated in the Vogel procedure (0.63–80.0 mg/kg), no significant activity of agomelatine or melatonin was detected in the USV model, although dose-dependent tendencies for a decrease in USV duration were observed with both compounds. In contrast, clorazepate decreased this response at a dose of 40.0 mg/kg. Over a broad dose-range (0.16–40.0 mg/kg), SB243,213 failed to decrease the duration of USV. ANOVA were as follows: agomelatine, F(4,41)=2.2, P>0.05; melatonin, F(4,39)=1.0, P>0.05; clorazepate, F(4,29)=3.7, P<0.05 and SB243,213, F(5,34)=0.2, P>0.05

Dialysis levels of serotonin and noradrenaline in the dorsal hippocampus and frontal cortex (Figs 6, 7, 8) Agomelatine dose-dependently (10.0–80.0 mg/kg) elevated extracellular levels of NA in the dorsal hippocampus of freely moving rats. ANOVA were as follows. 10.0 mg/kg, F(1,10)=3.2, P>0.05; 40.0 mg/kg, F(1,13)=5.0, P<0.05 and 80.0 mg/kg, F(1,9)=27.8, P<0.01. In distinction, melatonin (40.0 mg/kg and 80.0 mg/kg) failed to modify levels of NA either in the FCX (Millan et al. 2003) or in the hippocampus (Fig. 6). ANOVA was as follows. Melatonin, hippocampus, 80.0 mg/kg, F(1,10)=3.0, P>0.05. Neither agomelatine nor melatonin affected levels of 5-HT in the hippocampus and FCX (Fig. 6). ANOVA were as follows. Agomelatine, dorsal hippocampus; 5-HT, 10.0 mg/kg, F(1,10)=0.1, P>0.05; 40.0 mg/kg, F(1,12)=0.1, P>0.05 and 80.0 mg/kg, F(1,9)=4.6, P>0.05. Agomelatine, frontal cortex; 5-HT, 40.0 mg/kg, F(1,10)=0.5, P>0.05. Melatonin, dorsal hippocampus, 5-HT, 80.0 mg/kg, F(1,9)=0.3, P>0.05 and melatonin, frontal cortex; 5-HT, 40.0 mg/kg, F(1,10)=0.9, P>0.05. In contrast to agomelatine, clorazepate dose-dependently (0.63–20.0 mg/kg) and markedly decreased dialysis levels of NA and 5-HT both in the hippocampus and in the FCX

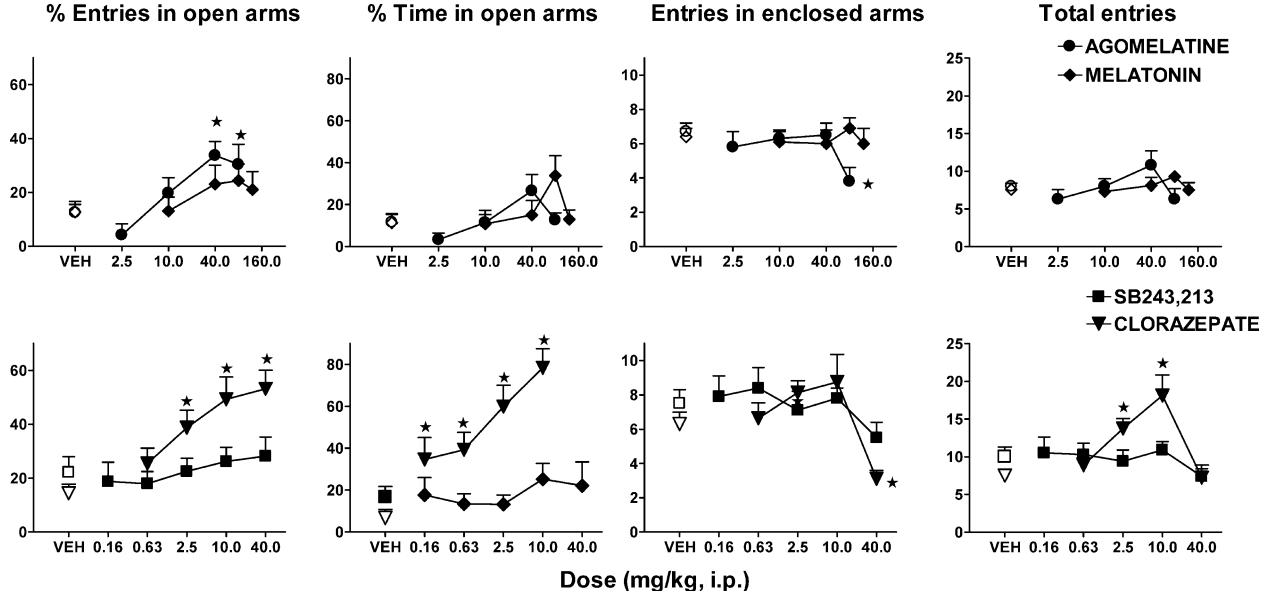

Fig. 4 Actions of agomelatine, melatonin, clorazepate and SB243,213 in the plus-maze test in rats. *VEH* vehicle. Data are means±SEMs. n=6–8 per value. *Asterisks* indicate significance of differences to respective vehicle values in Dunnett's test. *P<0.05

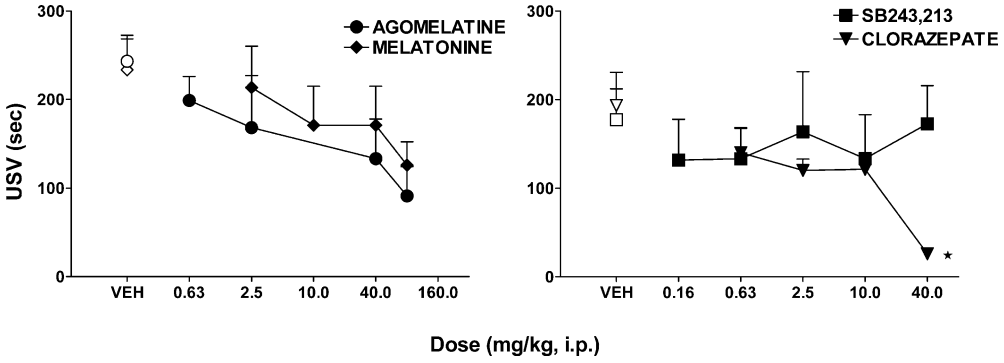

Fig. 5 Actions of agomelatine, melatonin, clorazepate and SB243,213 in the ultrasonic vocalization test in rats. *VEH* vehicle. Data are means±SEM. $n \geq 6$ per value. For ANOVA, see Results. The *asterisk* indicates significance of differences to vehicle values in Dunnett's test. *$P<0.05$

(Figs 7, 8). Clorazepate, dorsal hippocampus, 5-HT: 0.63 mg/kg, $F(1,9)=1.8$, $P>0.05$; 2.5 mg/kg, $F(1,11)=18.3$, $P<0.01$; 10.0 mg/kg, $F(1,11)=28.2$, $P<0.01$ and 20.0 mg/kg, $F(1,10)=45.4$, $P<0.01$ and clorazepate, dorsal hippocampus, NA: 0.63 mg/kg, $F(1,10)=0.7$, $P>0.05$; 2.5 mg/kg, $F(1,11)=0.7$, $P>0.05$; 10.0 mg/kg, $F(1,11)=32.0$, $P<0.01$ and 20.0 mg/kg, $F(1,10)=24.5$, $P<0.01$. Clorazepate, frontal cortex, 5-HT: 0.63 mg/kg, $F(1,11)=0.3$, $P>0.05$; 10.0 mg/kg, $F(1,12)=70.7$, $P<0.001$ and 20.0 mg/kg, $F(1,10)=47.7$, $P<0.01$ and clorazepate, frontal cortex, NA: 0.63, $F(1,11)=0.2$, $P>0.05$; 10.0 mg/kg, $F(1,12)=17.2$, $P<0.01$ and 20.0 mg/kg, $F(1,10)=20.5$, $P<0.01$. Mimicking the effect of agomelatine, SB243,213 provoked a significant and dose-dependent increase in extracellular levels of NA, but not of 5-HT, in the FCX (0.63–10.0 mg/kg) and hippocampus (10–20 mg/kg) (Figs 7, 8). SB243,213, dorsal hippocampus, 5-HT: 10.0 mg/kg, $F(1,11)=0.1$, $P>0.05$; 15.0 mg/kg, $F(1,11)=1.8$, $P>0.05$ and 20.0 mg/kg, $F(1,12)=1.1$, $P>0.05$ and SB243,213, dorsal hippocampus, NA: 10.0 mg/kg, $F(1,11)=0.9$, $P>0.05$; 15.0 mg/kg, $F(1,11)=6.7$, $P<0.05$ and 20.0 mg/kg, $F(1,12)=29.8$, $P<0.01$. SB243,213, frontal cortex, 5-HT: 0.63 mg/kg, $F(1,9)=0.3$, $P>0.05$; 2.5 mg/kg, $F(1,10)=0.3$, $P>0.05$ and 10.0 mg/kg, $F(1,11)=2.2$, $P>0.05$ and SB243,213, frontal cortex, NA: 0.63 mg/kg, $F(1,9)=0.3$, $P>0.05$; 2.5 mg/kg, $F(1,10)=13.5$, $P<0.01$ and 10.0 mg/kg, $F(1,11)=25.5$, $P<0.01$.

Motor behaviour (Fig. 9) Agomelatine elicited a dose-dependent reduction in spontaneous locomotor activity in rats. These actions were statistically significant at doses of 40.0 mg/kg and 80.0 mg/kg, respectively. Similarly,

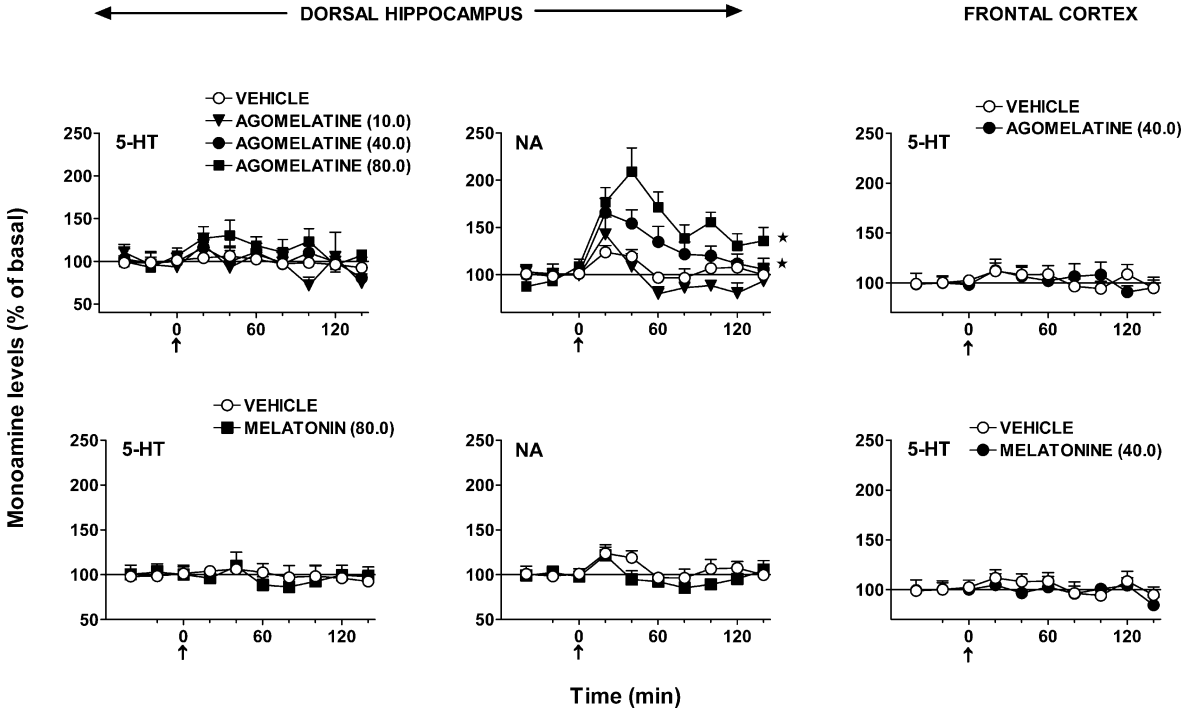

Fig. 6 Influence of agomelatine and melatonin on extracellular levels of serotonin (*5-HT*) and noradrenaline (*NA*) in the dorsal hippocampus, and of 5-HT in the frontal cortex, of freely moving rats. The *upper panels* represent the actions of agomelatine and the *lower panels* represent the actions of melatonin. Data are means ±SEMs. n=5–9 per value. Basal levels of 5-HT and NA in the dorsal hippocampus were 0.70±0.06 and 0.53±0.03 pg/20 µl, respectively. Basal levels of 5-HT in the frontal cortex were 0.61±0.03 pg/20 µl. For ANOVA, see Results. The *asterisks* indicate significance of differences between drug-treated and vehicle-treated groups. *$P<0.05$

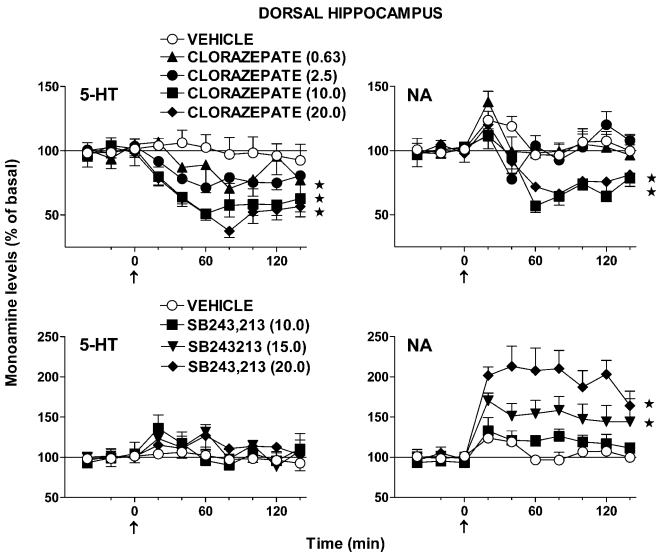

Fig. 7 Influence of clorazepate and SB243,213 on extracellular levels of serotonin (*5-HT*) and noradrenaline (*NA*) in the dorsal hippocampus of freely moving rats. The *upper panels* represent the actions of clorazepate and the *lower panels* represent the actions of SB243,213. Data are means±SEMs. *n*=5–8 per value. For ANOVA, see Results. *Asterisks* indicate significance of drug-treated groups versus vehicle-treated groups. **P*<0.05

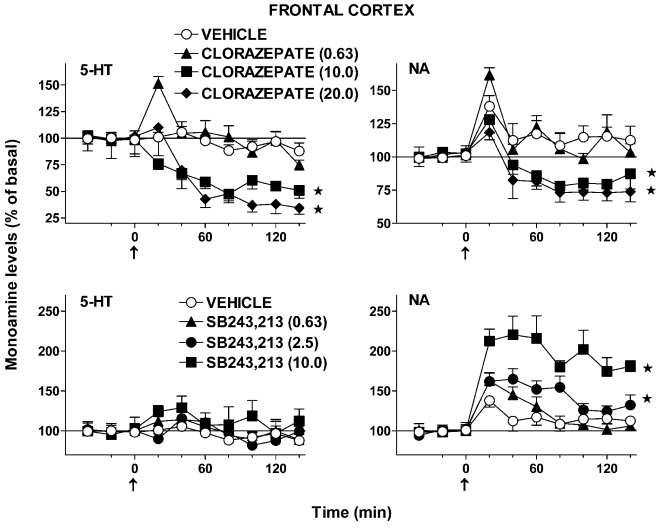

Fig. 8 Influence of clorazepate and SB243,213 on extracellular levels of serotonin (*5-HT*) and noradrenaline (*NA*) in the frontal cortex of freely moving rats. The *upper panels* represent the actions of clorazepate and the *lower panels* represent the actions of SB243,213. Data are means±SEMs. *n*=5–8 per value. For ANOVA, see Results. *Asterisks* indicate significance of drug-treated groups versus vehicle-treated groups. **P*<0.05

melatonin, clorazepate and SB243,213 all elicited dose-dependent reductions in locomotor activity in rats. ANOVA were as follows: agomelatine, $F(4,38)=13.8$, $P<0.001$; melatonin, $F(4,35)=10.9$, $P<0.001$; clorazepate, $F(5,34)=9.2$, $P<0.001$ and SB243,213, $F(4,29)=5.7$, $P<0.01$

Discussion

Anxiolytic profile of agomelatine as compared with clorazepate and SB243,213 In each model examined, clorazepate displayed significant anxiolytic activity, in line with extensive studies of the broad-based and robust anxiolytic properties of BZPs in rodents and pigeons (Sanger 1985; Miczek et al. 1995; Kleven and Koek 1999; Menard and Treit 1999; Millan and Brocco 2003). Nevertheless, the comparatively low potency of clorazepate in the USV procedure—exerted only at a dose which compromises motor function—coincides with previous work indicating that BZPs display modest activity in this model (Molewijk et al. 1995; Millan 2003).

Blockade of 5-HT_{2C} sites in the amygdala and hippocampus is associated with anxiolytic properties (Millan 2003) and the novel 5-HT_{2C} receptor antagonist, SB243,213, displayed significant anxiolytic properties in the social interaction test. This observation corroborates the findings of Wood et al. (2001) and parallels the actions of other 5-HT_{2C} receptor antagonists in this model (Kennett et al. 1996, 1997; Griebel et al. 1997; Dekeyne et al. 2000a; Millan et al. 2001; Millan and Brocco 2003). Wood et al. (2001) likewise reported significant activity of SB243,213 in a Geller–Seifter conflict paradigm. However, its effect was not dose-dependent and less pronounced than that of the BZP, diazepam. Moreover, SB243,213 showed only modest activity as compared with previous studies of other 5-HT_{2C} antagonists such as SB206,553 and SB242,084 (op. cit.). It is, thus, of particular interest that in the Vogel protocol, a related punishment-based conflict procedure (Dekeyne et al. 2000a; Millan et al. 2001; Millan and Brocco 2003), SB243,213 elicited a dose-dependent and robust increase in punished responses. On the other hand, reproducing findings with other 5-HT_{2C} receptor antagonists (Sànchez and Mørk 1999; Millan et al. 2001; Millan 2003), SB243,213 was ineffective in the USV procedure. This is not surprising since the USV paradigm mimics "panic-like states" which are abrogated by activation rather than blockade of 5-HT_{2C} sites, probably localized in the periaqueductal grey (Deakin 1994; Molewijk et al. 1995; Graeff et al. 1996; Jenck et al. 1998; Millan 2003). Finally, anxiolytic actions of 5-HT_{2C} antagonists in the plus-maze model have generally proven weak and variable (Griebel et al. 1997; Millan et al. 2001; Millan 2003), corresponding to the lack of efficacy of SB243,213 herein.

Agomelatine shared the anxiolytic profile of SB243,213 in that it was active in the social interaction and Vogel paradigms, but not in the USV procedure. By analogy to SB243,213, doses of agomelatine required to increase punished responses in the Vogel procedure were substantially higher than those active in the social interaction model. This observation probably reflects a difference in "test sensitivity" inasmuch as relatively high doses of anxiolytic agents, irrespective of their mechanistic class, are needed to obtain robust and significant effects in the Vogel procedure (Graeff et al. 1996; Menard and Treit 1999; Millan and Brocco 2003). On the other hand,

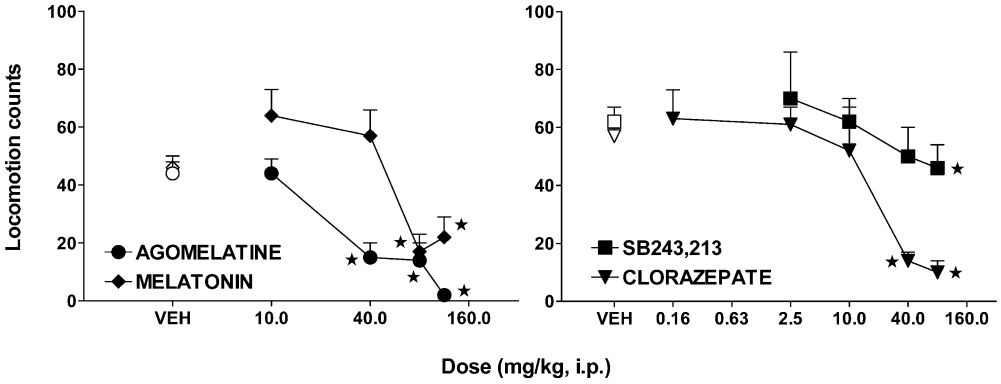

Fig. 9 Influence of drugs on spontaneous locomotion in rats. *VEH* vehicle. Data are means ±SEMs. n=5–8 per value. For ANOVA, see Results. *Asterisks* indicate significance of differences to vehicle values in Dunnett's test following ANOVA. *P<0.05

inasmuch as Sprague-Dawley rats were employed for the social interaction procedure as compared to Wistar rats for the Vogel test, a possible strain difference cannot be excluded: though we are not aware of data directly supporting this possibility, strain and genetic differences in the response of rats to anxiety and stress have been well documented (Belzung 2001; Clément et al. 2002; Millan 2003). It is also conceivable that the population of 5-HT_{2C} receptors which transduces anxiolytic properties in the Vogel test may differ to that involved in the social interaction procedure (Millan 2003). Interestingly, in contrast to SB243,213, agomelatine was effective in the plus-maze model. However, significant anxiolytic activity was seen only for two parameters (% entries in open arms and number of entries in enclosed arms) and for two and one doses, respectively, so this action would benefit from further evaluation in related models of anxiolytic activity.

Though agomelatine exerted a suppressive influence on spontaneous motor activity, agomelatine induced increases in active social interaction, of punished responses in the Vogel test and of open-arm entries in the plus-maze can hardly be attributed to a decrease in motor function. Further, melatonin similarly suppressed motor behaviour, yet failed to reveal anxiolytic properties. Indeed, the inhibitory influence of high doses of agomelatine on motor function is more likely to underlie an apparent "loss" of anxiolytic actions (dose–response curve inflection) in the social interaction and plus-maze paradigms.

Serotonergic transmission Extending our studies of the inhibitory influence of diazepam and chlordiazepoxide on corticolimbic release of 5-HT (Dekeyne et al. 2000a; Millan et al. 2001), clorazepate reduced levels of 5-HT both in the dorsal hippocampus and in the FCX. This $GABA_A$ receptor-mediated, suppressive influence of BZPs on serotonergic transmission is strongly implicated in their anxiolytic properties (Graeff et al. 1996; Menard and Treit 1999; Millan 2003). In contrast to BZPs, 5-HT_{2C} receptor antagonists do not modulate 5-HT release in corticolimbic structures (Gobert et al. 2000; Millan et al. 2000b) and, accordingly, SB243,213 failed to modify extracellular levels of 5-HT in FCX or dorsal hippocampus. Though it has been proposed that high doses of melatonin modulate cerebral 5-HT release under certain conditions, data remain fragmentary (Chuang and Lin 1994; Yoshioka et al. 2000) and no effect of melatonin on extracellular levels of 5-HT was observed herein. In line with this lack of influence of SB243,213 and melatonin on 5-HT levels, they were not affected by agomelatine. In distinction to BZPs, then, modulation of 5-HT release is not involved in the anxiolytic properties of agomelatine (Table 1). In light of evidence that mechanisms underlying the anxiolytic profile of agomelatine differ to those harnessed by clorazepate, it is of interest that agomelatine does not generalize to a discriminative stimulus elicited by a further BZP, diazepam, in rats (Wiley et al. 1998).

Adrenergic transmission The present study extends our previous observations of the inhibitory influence of chlordiazepoxide on dialysis levels of NA in the FCX (Millan et al. 2001) in demonstrating a similar suppressive influence of clorazepate both in this structure and in the dorsal hippocampus. These observations support the notion that a reduction of adrenergic transmission participates in the anxiolytic actions of BZPs (Tanaka et al. 2000; Millan 2003). In distinction to clorazepate, SB243,213 elevated extracellular levels of NA in the FCX and dorsal hippocampus, actions reflecting relief of the tonic, excitatory influence of 5-HT_{2C} receptors on GABAergic interneurones in the locus coeruleus (Gobert et al. 2000; Millan et al. 2000b). In contrast to melatonin, via blockade of 5-HT_{2C} receptors, agomelatine also enhanced extracellular levels of NA in hippocampus, mimicking its elevation of NA levels in the FCX (Millan et al. 2003). These observations reveal, then, a fundamental difference in the neurochemical actions of agomelatine versus BZPs of relevance to their contrasting profiles of anxiolytic activity. The increase in extracellular levels of NA evoked by agomelatine may be related, by analogy to other drugs acting as 5-HT_{2C} antagonists, to its lack of efficacy in the USV model (vide supra) inasmuch as panic attacks have been linked to an abrupt acceleration of the corticolimbic release of NA (Gorman et al. 2000; Millan 2003). On the other hand, in distinction to BZPs, a reinforcement of cortical adrenergic transmission by agomelatine and 5-HT_{2C} receptor antagonists may exert a positive influence on cognitive-attentional function and depressed mood (Foote and Aston-Jones 1995; Millan et al. 2000b).

Mechanisms underlying anxiolytic properties of agomelatine: importance of 5-HT$_{2C}$ receptor blockade As mentioned above, the anxiolytic and neurochemical profile of agomelatine resembles that of SB243,213 and other 5-HT$_{2C}$ receptor antagonists (Gobert et al. 2000; Millan 2003; Millan et al. 2003). Several other lines of evidence also support a role of 5-HT$_{2C}$ receptor blockade rather than agonist properties at melatoninergic sites in the anxiolytic actions of agomelatine. First, agomelatine displays antagonist properties at native, rat and cloned, human 5-HT$_{2C}$ receptors in vitro (Millan et al. 2003). Second, active doses of agomelatine herein correspond well to those at which it expresses its 5-HT$_{2C}$ antagonist properties in other models in vivo (Chagraoui et al. 2003; Millan et al. 2003). Third, though agomelatine possesses antagonist properties at 5-HT$_{2B}$ receptors, their blockade is unlikely to be involved in its anxiolytic actions inasmuch as SB243,213 has low affinity for these sites, while the selective 5-HT$_{2C}$ versus 5-HT$_{2B}$ antagonist, SB242,084, is active in the social interaction and Vogel procedures (Kennett et al. 1997; Millan et al. 2001). Moreover, selective 5-HT$_{2B}$ antagonists are ineffective in these procedures and stimulation of 5-HT$_{2B}$ sites is associated with anxiolytic properties (Kennett et al. 1998; Millan 2003).

Arguing against a role of melatonin receptors, administered at a dose which blocks the chronobiotic actions of agomelatine and other melatonin agonists (Weibel et al. 1999; Kopp et al. 1999b), the MT$_1$/MT$_2$ antagonist, S22153, failed to modify the anxiolytic properties of agomelatine. Moreover, melatonin did not display anxiolytic properties in the models employed herein, though its suppression of social interaction at a high dose presumably reflects sedative rather than anxiogenic properties (see above). While actions of melatonin in the social interaction and USV procedures have not previously been documented, melatonin has been reported to show anxiolytic activity in a plus-maze procedure in rats (Golombek et al. 1993). However, this action was only expressed at night and, in line with the present findings, it was inactive during the day. Similarly, while Naranjo-Rodriguez et al. (2000) documented anxiolytic actions of low doses of melatonin (0.5–2.0 mg/kg) in a rat Vogel conflict procedure, these studies were likewise performed during the dark part of a reversed 12 h light/dark cycle. Procedural variables, such as a short period of water deprivation, may account for these surprising results inasmuch as no other reports of anxiolytic actions of melatonin in conflict models are available (Millan and Brocco 2003).

General discussion Several general aspects of this study require brief commentary.

First, the major aim was to characterize the role of 5-HT$_{2C}$ receptor blockade in the potential anxiolytic actions of agomelatine. It would be of interest to extend the present work to certain (unusual) procedures in which anxiolytic properties of melatonin and other agonists at MT$_1$/MT$_2$ receptors have been expressed: for example, the anxious behaviour associated with "sickness" in rats (Kopp et al. 1999a,b, 2000; Nava and Carta 2001).

Second, while the present evidence support a role of 5-HT$_{2C}$ receptor blockade in the anxiolytic actions of agomelatine (Table 1), their localization was not addressed. It is likely that populations in the hippocampus and amygdala are involved: this remains, however, to be demonstrated (Kennett et al. 1997; Menard and Treit 1999; Wood et al. 2001; Millan 2003).

Third, in the present and other (Millan, M.J. et al. 2003; unpub. obs.) procedures of 5-HT$_{2C}$ receptor-mediated activity in vivo, agomelatine was generally approximately 5-fold less potent than SB243,213. This modest difference in potency may seem surprising inasmuch as the affinity of agomelatine is some 100-fold lower than that of SB243,213 for cloned, human 5-HT$_{2C}$ receptors (Wood et al. 2001; Millan et al. 2003). One possibility is that the relative affinities of agomelatine and SB243,213 at cerebral rat 5-HT$_{2C}$ sites are different from those determined at heterologously expressed h5-HT$_{2C}$ sites. In this light, it is important to note that several, species-specific isoforms of 5-HT$_{2C}$ receptors have been described which display contrasting pharmacological profiles (Millan 2003). It might also be contended that the melatoninergic properties of agomelatine potentiate its 5-HT$_{2C}$ receptor-mediated effects in vivo. However, this argument is countered by the lack of influence of the melatoninergic antagonist, S22153, on the actions of agomelatine (Fig. 3 and Millan et al. 2003). It should also be noted that SB243,213 is less potent in vivo than might be expected from a comparison with the first-generation and chemically related 5-HT$_{2C}$ receptor antagonist, SB200,646: though SB243,213 is 100-fold more potent than the latter agent, they are of similar potency in vivo (Kennett et al. 1994; Wood et al. 2001; Millan M.J. et al., unpublished data). Finally, though it might be argued that bioactive metabolites may contribute to in vivo actions of agomelatine and SB243,213, there is currently no evidence for this possibility and pharmacokinetic factors are generally of lesser importance for parenteral routes of injection (as used herein) than for oral administration. Thus, the question of the relative potencies in vivo of agomelatine relative to SB243,213 and other 5-HT$_{2C}$ receptor antagonists requires further elucidation.

Finally, the suprachiasmatic nucleus, a major site of action of melatonin (Ying et al. 1996; Liu et al. 1997), possesses a high concentration of 5-HT$_{2C}$ receptors (Roca et al. 1993). Their activation participates in the influence of light on pineal secretion of melatonin (Kennaway and Moyer 1998, 1999; Kennaway et al. 2001; Hay-Schmidt et al. 2003). Whether antagonist properties of agomelatine at 5-HT$_{2C}$ sites controlling melatonin release are relevant to its influence on anxious states remains to be evaluated.

Conclusions In conclusion, the novel melatonin agonist/5-HT$_{2C}$ receptor antagonist, agomelatine, displayed a pattern of anxiolytic activity resembling that of the selective 5-HT$_{2C}$ receptor antagonist, SB243,213 (Table 1). Further, in the models examined herein, melatonin was inactive and anxiolytic actions of agomelatine were resistant to the MT$_1$/MT$_2$ antagonist, S22153. Thus, under the present

conditions, 5-HT_{2C} receptor blockade underlies the anxiolytic properties of agomelatine. Accordingly, the anxiolytic profile of agomelatine differed from that of the BZP, clorazepate, from which it could also be distinguished by its lack of inhibitory influence on corticolimbic release of 5-HT and NA. It will be important to determine the relevance of these observations to the therapeutic utility of agomelatine in the treatment of depressive and, potentially, anxious states (Lôo et al. 2002b), both of which are associated with altered activity at cerebral populations of 5-HT_{2C} receptors (Deakin 1994; Millan 2003). Underpinning interest in this question, agomelatine was recently found to suppress anxious symptoms in depressed patients (Lôo et al. 2002a) though studies in "pure" anxious subjects remain to be undertaken.

Acknowledgements The authors thank Laetitia Cistarelli, Brigitte Denorme, Huguette Gressier, Loretta Iob, Rodolphe Billiras, Christophe Melon and Jimmy Mullot for technical assistance, and Marianne Soubeyran for secretarial assistance. E. Mocäer is thanked for helpful comments on the manuscript.

References

Atsmon J, Oaknin S, Laudon M, Laschiner S, Gavish M, Dagan Y, Zisapel N (1996) Reciprocal effects of chronic diazepam and melatonin on brain melatonin and benzodiazepine binding sites. J Pineal Res 20:65–71

Belzung C (2001) The genetic basis of the pharmacological effects of anxiolytics: a review based on rodent models. Behav Pharmacol 12:451–460

Borjigin J, Li X, Snyder SH (1999) The pineal gland and melatonin: molecular and pharmacologic regulation. Annu Rev Pharmacol Toxicol 39:53–65

Bristow LJ, O'Connor D, Watts R, Duxon MS, Hutson PH (2000) Evidence for accelerated desensitisation of 5-HT_{2C} receptors following combined treatment with fluoxetine and the 5-HT_{1A} receptor antagonist, WAY100,635, in the rat. Neuropharmacology 39:1222–1236

Cardinali DP, Gvozdenovich E, Kaplan MR, Fainstein I, Shifis HA, Pérez Lloret S, Albornoz L, Negri A (2002) A double blind-placebo controlled study on melatonin efficacy to reduce anxiolytic benzodiazepine use in the elderly. Neuroendocrinol Lett 23:55–60

Casacalenda N, Boulenger JP (1998) Pharmacologic treatment effective in both generalized anxiety disorder and major depressive disorder: clinical and theoretical implications. Can J Psychiatry 43:722–730

Cervo L, Samanin R (1995) 5-HT_{1A} receptor full and partial agonists and 5-HT_{1C} (but not 5-HT_3) receptor antagonists increase rates of punished responding in rats. Pharmacol Biochem Behav 52:671–676

Chagraoui A, Protais P, Filloux T, Mocaër E (2003) Agomelatine (S20098) antagonizes the penile erections induced by the stimulation of 5-HT_{2C} receptors in Wistar rats. Psychopharmacology 170:17–22

Chuang IL, Lin MT (1994) Pharmacological effects of melatonin treatment on both locomotor activity and brain serotonin release in rats. J Pineal Res 17:11–16

Clemett DA, Punhani T, Duxon MS, Blackburn TP, Fone KCF (2000) Immunohistochemical localisation of the 5-HT_{2C} receptor protein in the rat CNS. Neuropharmacology 39:123–132

Clément Y, Calatayud F, Belzung C (2002) Genetic basis of anxiety-like behaviour: a critical review. Brain Res Bull 57:57–71

Das S, Tecott L (1996) Diminished anxiety-like responses in 5-HT_{2C} receptor mutant mice. Soc Neurosci Abstr 22:811–812

Deakin JFW (1994) Three distinct roles of 5-HT in anxiety, panic and depression. In: Montgomery SA, Corn TH (eds) Psychopharmacology of depression. British Association for Psychopharmacology, Cambridge, vol 13, pp 87–101

Dekeyne A, Brocco M, Adhumeau A, Gobert A, Millan MJ (2000a) The selective serotonin 5-HT_{1A} receptor ligand, S15535, displays anxiolytic-like effects in the social interaction and Vogel models and suppresses dialysate levels of 5-HT in the dorsal hippocampus of freely-moving rats: a comparison with other anxiolytic agents. Psychopharmacology 152:55–66

Dekeyne A, Denorme B, Monneyron S, Millan MJ (2000b) Citalopram reduces social interaction in rats by activation of serotonin (5-HT)$_{2C}$ receptors. Neuropharmacology 39:1114–1117

File SE, Seth P (2003) A review of 25 years of the social interaction test. Eur J Pharmacol 463:35–53

Foote SL, Aston-Jones GS (1995) Pharmacology and physiology of central noradrenergic systems. In: Bloom FE, Kupfer DJ (eds) Psychopharmacology: the fourth generation in progress. Raven, New York, pp 347–354

Gobert A, Rivet J-M, Lejeune F, Newman-Tancredi A, Adhumeau-Auclair A, Nicolas J-P, Cistarelli L, Melon C, Millan MJ (2000) Serotonin$_{2C}$ receptors tonically suppress the activity of mesocortical dopaminergic and adrenergic, but not serotonergic, pathways: a combined dialysis and electrophysiological analysis in the rat. Synapse 36:205–221

Golombek DA, Martini M, Cardinali DP (1993) Melatonin as an anxiolytic in rats: time dependence and interaction with the central GABAergic system. Eur J Pharmacol 237:231–236

Golombek DA, Pevet P, Cardinali DP (1996) Melatonin effects on behavior: possible mediation by the central GABAergic system. Neurosci Biobehav Res 20:403–412

Gorman JM, Kent JM, Sullivan GM, Coplan JD (2000) Neuroanatomical hypothesis of panic disorder, revised. Am J Psychiatry 157:493–505

Graeff FG, Guimaraes FS, De Andrade TGCS, Deakin JFW (1996) Anxiety and role of 5-HT in stress and anxiety and depression. Pharmacol Biochem Behav 54:129–141

Griebel G, Perrault G, Sanger DJ (1997) A comparative study of the effects of selective and non-selective 5-HT_2 receptor subtype antagonists in rat and mouse models of anxiety. Neuropharmacology 36:793–802

Guardiola-Lemaitre B, Lenegre A, Porsolt RD (1992) Combined effects of diazepam and melatonin in two tests for anxiolytic activity in the mouse. Pharmacol Biochem Behav 41:405

Hay-Schmidt A, Vrang N, Larsen PJ, Mikkelsen JD (2003) Projections from the raphe nuclei to the suprachiasmatic nucleus of the rat. J Chem Neurosci 25:293–310

Jenck F, Bös M, Wichmann J, Stadler H, Martin JR, Moreau JL (1998) The role of 5-HT_{2C} receptors in affective disorders. Exp Opin Invest Drugs 7:1587–1599

Kennaway DJ, Moyer RW (1998) Serotonin 5-HT_{2C} agonists mimic the effect of light pulses on circadian rhythms. Brain Res 806:257–270

Kennaway DJ, Moyer RW (1999) MK-801 administration blocks the effects of a 5-$HT_{2A/2C}$ agonist on melatonin rhythmicity and c-fos induction in the suprachiasmatic nucleus. Brain Res 845:102–106

Kennaway DJ, Moyer RW, Voultsios A, Varcoe TJ (2001) Serotonin, excitatory amino acids and the photic control of melatonin rhythms and SCN c-fos in the rat. Brain Res 897:36–43

Kennett GA, Wood MD, Glen A, Grewal S, Forbes I, Gadre A, Blackburn TP (1994) In vivo properties of SB 200646A, a 5-$HT_{2C/2B}$ receptor antagonist. Br J Pharmacol 111:797–802

Kennett GA, Wood MD, Bright F, Cilia J, Piper DC, Gager T, Thomas D, Baxter GS, Forbes IT, Ham P, Blackburn TP (1996) In vitro and in vivo profile of SB 206553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. Br J Pharmacol 117:427–434

Kennett GA, Wood MD, Bright F, Trail B, Riley G, Holland V, Avenell KY, Stean T, Upton N, Bromidge S, Forbes IT, Brown AM, Middlemiss DN, Blackburn TP (1997) SB 242084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. Neuropharmacology 36:609–620

Kennett GA, Trail B, Bright F (1998) Anxiolytic-like actions of BW 723C86 in the rat Vogel conflict test are 5-HT_{2B} receptor mediated. Neuropharmacology 37:1603–1610

Kleven MS, Koek W (1999) Effects of benzodiazepine agonists on punished responding in pigeons and their relationship with clinical doses in humans. Psychopharmacology 141:206–212

Kopp C, Vogel E, Rettori MC, Delagrange P, Guardiola-Lemaître B, Misslin R (1999a) Effects of melatonin on neophobic responses in different strains in mice. Pharmacol Biochem Behav 63:521–526

Kopp C, Vogel E, Rettori MC, Delagrange P, Renard P, Lesieur D, Misslin R (1999b) Antagonistic effects of S22153, a new MT_1 and MT_2 receptor ligand, on the neophobia-reducing properties of melatonin in BALB/c mice. Pharmacol Biochem Behav 64:131–136

Kopp C, Vogel E, Rettori MC, Delagrange P, Misslin R (2000) Anxiolytic-like properties of melatonin receptor agonists in mice: involvement of MT_1 and/or MT_2 receptors in the regulation of emotional responsiveness. Neuropharmacology 39:1865–1871

Krueger KE (1991) Peripheral-type benzodiazepine receptors: a second site of action for benzodiazepines. Neuropharmacology 4:237–244

Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, Reppert SM (1997) Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. Neuron 19:91–102

Lôo H, D'haenen H, Hale A (2002a) A double-blind study of S20098 in patients with major depressive or bipolar II disorders: effect on anxiety. Int J Neuropsychopharmacol 5: P3.E.031

Lôo H, Hale A, D'haenen H. (2002b) 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

Lopez-Gimenez J, Tecott LH, Palacios JM (2002) Serotonin 5-HT_{2C} receptor knockout mice: autoradiographic analysis of multiple serotonin receptors. J Neurosci Res 67:69–85

Menard J, Treit D (1999) Effects of centrally administered anxiolytic compounds in animal models of anxiety. Neurosci Biobehav Rev 23:591–613

Miczek KA, Weerts E, Vivian JA, Barros HM (1995) Aggression, anxiety and vocalizations in animals: $GABA_A$ and 5-HT anxiolytics. Psychopharmacology 121:38–56

Millan MJ (2003) The neurobiology and control of anxious states. Prog Neurobiol 70:53–244

Millan MJ, Brocco M (2003) The Vogel conflict test: procedural aspects, γ-aminobutyric acid, glutamate and monoamines. Eur J Pharmacol 463:67–96

Millan MJ, Gobert A, Rivet JM, Adhumeau-Auclair A, Cussac D, Newman-Tancredi A, Dekeyne A, Nicolas JP, Lejeune F (2000a) Mirtazapine enhances frontocortical dopaminergic and corticolimbic adrenergic, but not serotonergic, transmission by blockade of α_2-adrenergic and serotonin$_{2C}$ receptors: a comparison with citalopram. Eur J Neurosci 12:1079–1095

Millan MJ, Lejeune F, Gobert A (2000b) Reciprocal autoreceptor and heteroreceptor control of serotonergic, dopaminergic and noradrenergic transmission in the frontal cortex: relevance to the actions of antidepressant agents. J Psychopharmacol 14:114–138

Millan MJ, Brocco M, Gobert A, Dorey G, Casara P, Dekeyne A (2001) Anxiolytic properties of the selective, non-peptidergic CRF_1 antagonists, CP154,526 and DMP695: a comparison to other classes of anxiolytic agent. Neuropsychopharmacology 25:585–600

Millan MJ, 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-hydroxytryptamine$_{2C}$ receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. J Pharmacol Exp Ther 306:954–964

Molewijk HE, van der Poel AM, Mos J, van der Heyden JAM, Olivier B (1995) Conditioned ultrasonic distress vocalizations in adult male rats as a behavioural paradigm for screening anti-panic drugs. Psychopharmacology 117:32–40

Naguib M, Samarkandi AH (2000) The comparative dose–response effects of melatonin and midazolam for premedication of adult patients: a double-blinded, placebo-controlled study. Anesth Analg 91:473–479

Naranjo-Rodriguez EB, Ortiz Orsornio A, Hernandez-Avitia E, Mendoza-Fernandez V, Escobar A (2000) Anxiolytic-like actions of melatonin, 5-methoxytryptophan, 5-hydroxytryptophol and benzodiazepines on a conflict procedure. Prog Neuropsychopharmacol Biol Psychiatr 24:117–129

Nava F, Carta G (2001) Melatonin reduces anxiety induced by lipopolysaccharide in the rat. Neurosci Lett 307:57–60

Niles L (1991) Melatonin interaction with the benzodiazepine-GABA receptor complex in the CNS. Adv Exp Med Biol 294:267–277

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

Pierrefiche G, Zerbib R, Laborit H (1993) Anxiolytic activity of melatonin in mice: involvement of benzodiazepine receptors. Res Commun Chem Pathol Pharmacol 82:131–142

Roca AL, Weaver DR, Reppert SM (1993) Serotonin receptor gene expression in the rat suprachiasmatic nuclei. Brain Res 608:159–165

Rocha B, Rigo M, Di Scala G, Sandner G, Hoyer D (1994) Chronic mianserin or eltoprazine treatment in rats: effects on the elevated plus-maze test and on limbic 5-HT_{2C} receptor levels. Eur J Pharmacol 262:125–131

Sanger DJ (1985) GABA and the behavioural effects of anxiolytic drugs. Life Sci 36:1503–1513

Sánchez C (2003) Stress-induced vocalization in adult animals. A valid model of anxiety? Eur J Pharmacol 463:133–143

Sànchez C, Mørk A (1999) *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline studies on the role of 5-HT_{1A} and 5-HT_2 receptors in mediating foot-shock-induced ultrasonic vocalisation in adults rats. Eur Neuropsychopharmacol 9:287–294

Tanaka M, Yoshida M, Emoto H, Ishii H (2000) Noradrenaline systems in the hypothalamus, amydgala and locus coeruleus are involved in the provocation of anxiety: basic studies. Eur J Pharmacol 405:397–406

Van Reeth O, Weibel L, Olivares E, Maccari S, Mocaër E, Turek FW (2001) Melatonin or a melatonin agonist correct age-related changes in circadian response to an environmental stimulus. Am J Physiol 280:1582–1591

Wall PM, Messier C (2001) Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior. Neurosci Biobehav Rev 25:275–286

Weibel L, Retorri MC, Lesieur D, Delagrange P, Renard P, Van Reeth O (1999) A single oral dose of S22153, a melatonin antagonist, blocks the phase advancing effects of melatonin in C3H mice. Brain Res 829:160–166

Wiley JL, Dance ME, Balster BL (1998) Preclinical evaluation of the reinforcing and discriminative stimulus effects of agomelatine (S20098), a melatonin agonist. Psychopharmacology 140:503–509

Wood MD, Reavill C, Trail B, Wilson A, Stean T, Kennett GA, Lightowler S, Blackburn TP, Thomas D, Gager TL, Riley G, Holland V, Bromidge SM, Forbes IT, Middlemiss DN (2001) SB-243213; a selective 5-HT_{2C} receptor inverse agonist with improved anxiolytic profile: lack of tolerance and withdrawal anxiety. Neuropharmacology 41:186–199

Ying SW, Rusak B, Delagrange P (1996) Melatonin analogues as agonist and antagonists in the circadian system and other brain areas. Eur J Pharmacol 296:33–42

Yoshioka K, Xie F, Gitzen JF, Kissinger CB, Kissinger PT (2000) Preliminary study of the effects of melatonin administration on the release of endogenous 5-HT and its metabolite in rat SCN. Curr Separation 18:117–122

Yous S, Andrieux J, Howell HE, Morgan PJ, Renard P, Pfeiffer B, Lesieur D, Guardiola-Lemaître B (1992) Novel naphtalenic ligands with high affinity for the melatonin receptor. J Med Chem 35:1484–1585