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ORIGINAL INVESTIGATION

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Antidepressant-like effects of agomelatine, melatonin and the NK_1 receptor antagonist GR205171 in impulsive-related behaviour in rats

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Abstract *Rationale:* Substance P receptor [neurokinin$_1$ (NK_1-R)] antagonists and melatonin$_{1/2}$ receptor ($MT_{1/2}$-R) agonists have been claimed to be potential antidepressants (ADs). In animals, these compounds are active in validated models responsive to ADs, such as forced swimming test and chronic mild stress paradigms. Classical AD drugs are also known to be effective in pathologies characterized by an impulse control deficiency. In line with this clinical observation, previous studies demonstrated that classical ADs increased the capacity to wait for food reward in rats subjected to a paradigm aimed at assessing impulsive-related behaviour. *Objectives:* This study was conducted to investigate the effects of two $MT_{1/2}$-R agonists, melatonin and agomelatine, and a NK_1-R antagonist, GR205171, on tolerance to delay of food reward in rats. *Methods:* Fasting rats were trained in a T-maze and allowed to choose between two magnitudes of reward: immediate but small reward (two pellets) vs 25-s delayed but large reward (ten pellets). Under this alternative, vehicle-injected rats selected the large-but-delayed reinforcer in less than 40% of the trials. *Results:* Like the established ADs clomipramine (8 mg kg^{-1}, i.p.) and fluvoxamine (4 mg kg^{-1}, i.p.), melatonin (3 and 10 mg kg^{-1}, i.p.), agomelatine (10 and 30 mg kg^{-1}, i.p.) and GR205171 (30 mg kg^{-1} but not 10 mg kg^{-1}, s.c.) significantly increased the number of choices of the large-but-delayed reward. The effect of melatonin (3 mg kg^{-1}, i.p.) was not counteracted by the $MT_{1/2}$-R antagonist S22153 (40 mg kg^{-1}, i.p.) that exerted no effect on its own. *Conclusion:* These results suggest that $MT_{1/2}$-R agonists and NK_1-R antagonists enhance rats' tolerance to delay of gratification, an effect which may reflect their ability to improve impulse control. Further investigations are necessary to clarify the neurobiological mechanisms responsible for this effect.

Keywords NK_1 receptors · MT_1/MT_2 receptors · Substance P · Delay of reward · Impulse control · Antidepressant · Rat

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Introduction

Antidepressants (ADs) have been successfully used for several decades in the treatment of major affective disorders. To date, all these drugs including tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs), facilitate monoaminergic transmission. More recently, other compounds, which do not act directly through interactions with monoamines, have been claimed to possess antidepressant properties. In fact, neuroactive molecules such as melatonin and substance P also seem to be involved in mood disorders and are potential targets for non-monoamine-based antidepressant treatments (Kramer et al. 1998, 2004; Lôo et al. 2002a,b). Melatonin, a neurohormone synthesized in the pineal gland during the dark period, is agonist at two receptors, MT_1 and MT_2. Substance P, a neuropeptide that belongs to the family of tachykinins, acts preferentially through the neurokinin$_1$ receptor (NK_1-R).

Preclinical studies indicated that non-selective melatonin receptor ($MT_{1/2}$-R) agonists and NK_1-R antagonists had a positive action in animal models responsive to antidepressants. Indeed, both melatonin and agomelatine, a potent agonist at $MT_{1/2}$-R (and antagonist at serotoninergic 5-$HT_{2B/2C}$ receptors; Millan et al. 2003) (Table 1), have been reported to decrease the time spent immobile in rodents subjected to the forced swimming test (Overstreet et al. 1998; Shaji and Kulkarni 1998; Raghavendra et al. 2000;

Table 1 Binding affinities of melatonin and agomelatine at cloned human MT_1, MT_2 and 5-HT_2 receptor subtypes

pK_i values	hMT_1	hMT_2	h5-HT_{2A}	h5-HT_{2B}	h5-HT_{2C}
Melatonin	9.7	9.5	<5.0	5.2	<5.0
Agomelatine	10.0	9.9	5.4	6.6	6.2

From Audinot et al. (2003) and Millan et al. (2003)

Wong and Ong 2001; Bourin et al. 2004) and the tail suspension test (Prakhie and Oxenkrug 1998; Mantovani et al. 2003). In addition, these compounds counteracted the reduction of sucrose consumption and behavioural disturbances induced by chronic mild stress (Kopp et al. 1999b; Papp et al. 2003). Likewise, NK_1-R antagonists have been described to induce antidepressant-like effects in the forced swimming test (Rupniak et al. 2001; Zocchi et al. 2003; Dableh et al. 2005), the tail suspension test (Varty et al. 2003), and the chronic mild stress procedure (Papp et al. 2000). Furthermore, in humans, agomelatine (Lôo et al. 2002a,b) and two NK_1-R antagonists, MK-869 and L-759274 (Kramer et al. 1998, 2004), have been reported to alleviate major depressive disorders, as evaluated by the Hamilton Depression Scale.

In animals, most studies were conducted using standard paradigms, i.e. the forced swimming and the tail suspension tests. Such paradigms involve experimental conditions that normally provoke escape responses but that cannot be escaped from. However, another component seems relevant to the effects of ADs: their ability to improve impulse control. Indeed, ADs have been shown to be effective in pathologies characterized by an impulse control deficiency (Marks et al. 1980; Turner et al. 1985). Since impulsive subjects are often described as intolerant to delay of gratification, the capacity to wait for food reward has been proposed as an approach to impulsive-related behaviour in animals. For this purpose, rats can be trained in a T-maze and allowed to choose between two magnitudes of food reward, an immediate but small reward (two pellets) vs a 25-s delayed but large reward (ten pellets). Under this alternative, rats more often select the small immediate reward (Thiébot et al. 1985a). Interestingly, a variety of ADs have been shown to increase the number of choices of the large-but-delayed reward, suggesting an enhancement of rat's waiting capacities (Bizot et al. 1988). Further studies then provided support to the idea that this effect actually reflects the ability of these drugs to improve impulse control. In contrast, drugs can decrease tolerance to delay of gratification. In particular, under 15-s delay conditions, anxiolytics such as benzodiazepines and partial agonists at 5-HT_{1A} receptors (i.e. buspirone) were shown to reduce the proportion of choices directed towards the larger delayed reinforcer (Thiébot et al. 1985a; Bizot et al. 1999).

In this context, it was interesting to investigate whether atypical antidepressant drugs are also able to enhance rats' tolerance to delay of gratification. To achieve this aim, the effects on waiting capacity of melatonin and agomelatine, two $MT_{1/2}$-R agonists, and GR205171, an antagonist having nanomolar affinity for the rat NK_1-R (Gardner et al. 1996), were assessed in rats subjected to the T-maze procedure (Thiébot et al. 1985a). These compounds were tested at relatively high doses (3–10 mg kg^{-1} for melatonin, 10–30 mg kg^{-1} for agomelatine and GR205171), chosen in the range of those previously found to be active in in vivo studies devoted to assess an antidepressant potential in rodents (Rupniak et al. 2001; Papp et al. 2003; Bourin et al. 2004; Hutson et al. 2004).

Materials and methods

The experiments were carried out on male Wistar AF rats (CERJ, Le Genest St-Isle, France) weighing 220±10 g at the beginning of training. They were housed six per cage (40×40×18 cm) under standard laboratory conditions (lights on from 0730 to 1930 hours, room temperature 21±1°C), with drinking water freely available in the home cage. Except when otherwise specified, animals were restricted to 11 g of standard chow day^{-1} rat^{-1} during the week preceding the beginning of the training, and then 13 g day^{-1} rat^{-1} until the end of the experiments. Rats were subjected to daily saline intraperitoneal (i.p.) or subcutaneous (s.c.) injections over a period of at least 5 days before receiving the drug under study. Training and test sessions took place between 0930 and 1400 hours, 5 days per week. The experiments were conducted in agreement with the institutional guidelines for use of animals and their care, in compliance with national and international laws and policies (council directives no. 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions no. 75-116 to M.H. and no. 75-118 to M.H.T.).

Choice behaviour in a T-maze

The experiments were conducted according to the procedure described by Thiébot et al. (1985a).

Apparatus

The experiments were performed in two identical T-mazes made of opaque gray plastic tubing (internal diameter, 7 cm). Each maze consists of a starting runway (50 cm long) and two arms (50 cm long) giving access to a rectangular goal box (10×20×10 cm) at their extremities. Both arms were equipped with two removable plastic guillotine doors that could be inserted into vertical clefts. One of the goal boxes (left or right, depending on the rats) was constantly provided with the large reward (ten pellets, 45 mg, Rodent Formula F0165, Bioserv Inc., Frenchtown, NJ, USA), the other with the small reward (two pellets). The pellets were placed in a translucent food cup before each trial.

Training procedure

Rats were first trained (one daily session of five trials, 3-min intertrial interval, kept constant throughout the training and testing phases) to traverse the same T-maze and to choose between the arm giving access to the small reward (two pellets) and the other arm giving access to the large reward (ten pellets). In no case were the rats able to visit the two arms during one single trial, and they were never subjected to forced-choice trials. Within about ten sessions, all the rats selected in more than 80% of the trials the arm giving access to the large reward. The second stage of training was then initiated. During this stage, when rats chose the arm associated with the large reward, they were detained in this arm (between the two guillotine doors) for a 25-s period before gaining access to the ten pellets. No delay was imposed before access to the small reward. Within four or five additional sessions, the choice behaviour stabilized at a new level and the rats selected the large (now 25-s delayed) reward in less than 40% of the trials.

Testing procedure

The testing phase was conducted over four consecutive daily sessions of five trials each. For that purpose, the rats were assigned to separate subgroups matched according to the number of trials in which they chose the large reward. They were given saline before each of the first two sessions (baseline sessions) and the drug under study, at the same dosage, before each of the last two sessions. Each dose of a given drug was tested on a different group of drug-naive rats (i.e. rats were not subjected to several drug tests). Control animals were injected with the vehicle under the same conditions as those used for the drug testing. The number of choices of the large-but-delayed reward was recorded for each subject.

Additional experiments

T-maze paradigm Two independent groups of rats were subjected to the T-maze paradigm under different experimental conditions. Rats of one group were tested under no delay conditions, i.e. at the end of the initial phase of training. Rats of the second group were trained as described above, but the access to the ten pellets was delayed by only 15 s during the second training stage and the testing phase. After four training sessions under these conditions, rats still selected the large-but-now-15 s-delayed reward in more than 80% of the trials. They were then subjected to the four test sessions as described above.

Food intake Drug- and test-naive male Wistar rats (300–340 g at the time of testing) were food-deprived for 16 h before being placed for a 30-min session in individual boxes (43×30×18 cm) covered by a grid, where they were provided with food. The food consisted of powdered usual rat chow mixed with water (2:1, v:v) and was placed in a glass cup. The quantity of food consumed was assessed by weighing the cup before and after the test session. Treatments with melatonin (3–10 mg kg^{-1}), agomelatine (10–30 mg kg^{-1}), and diazepam (2 mg kg^{-1}) used as positive control, or their respective vehicle, were randomly distributed across the rats.

Drugs

Melatonin (Sigma, Saint Quentin Fallavier, France), *N*-[2-(5-ethyl-benzo[*b*]thien-3-yl)ethyl]acetamide (S22153), a mixed $MT_{1/2}$-R antagonist (Institut de Recherches Internationales Servier, Courbevoie, France), 2-methoxy-5-(5-trifluoromethyl-tetrazol-1-yl)-(2-phenyl-piperidin-3-yl) amine (GR205171; GlaxoSmithKline, Stevenage, UK), and diazepam (Hoffmann La-Roche, Basel, Switzerland), used as positive control, were prepared by dispersion in saline (0.9% NaCl) with one drop of Tween 80. Agomelatine (S20098; Institut de Recherches Internationales Servier) was suspended in a 1% solution of hydroxyethylcellulose (HEC). Clomipramine HCl (Novartis Pharma, Basel, Switzerland) and fluvoxamine maleate (Solvay-Duphar, Lyon, France), used as positive controls, were dissolved in saline. The doses are expressed as base or salt, as appropriate. Drugs or vehicle were administered in the morning, between 0900 and 1400 hours, either i.p. (melatonin, agomelatine, S22153, clomipramine, fluvoxamine and diazepam) or s.c. (GR205171), 30 min, or 35 min for S22153, before the test sessions, in a volume of 5 ml kg body $weight^{-1}$.

Statistical analyses

Statistical comparisons between the mean total number of choices of the large-but-delayed reward during the two saline sessions vs the two drug (or vehicle) sessions were performed using two-tailed Student's *t* test for paired comparisons.

The quantity of food consumed (expressed as mg g body $weight^{-1}$) was analyzed by one-way analysis of variance (ANOVA), or two-tailed Student's *t* test for the diazepam group.

Results

Effect of melatonin

In control rats, the number of choices of the arm giving access to the large but 25-s delayed reward did not differ between the two baseline (saline) sessions and the two vehicle sessions (t=0.76, *NS*). Melatonin (3 and 10 mg kg^{-1}) increased the number of choices of the large-but-delayed reward (t=4.18, P<0.005 and t=3.17, P<0.01, respectively). Positive control rats, given fluvoxamine (4 mg kg^{-1}), also increased their choice of the large-but-delayed reward (t=2.32, P<0.05). Interestingly, the effects of melatonin, at the

two doses tested, were in the same range as that of fluvoxamine (Fig. 1a). When the delay was set at only 15 s, saline-injected rats choose the large-but-delayed reward in more than 80% of the trials (baseline), and this choice did not significantly differ during the two test sessions, after the administration of melatonin (3 mg kg^{-1}: t=0.66; 10 mg kg^{-1}: t=1.31, *NS*) or vehicle (t=1.67, *NS*). Positive control rats, given diazepam (2 mg kg^{-1}), significantly reduced the number of choices of the large-but-15 s-delayed reward (t=3.80, P<0.01) (Fig. 1b). When no delay was introduced before access to the ten pellets, melatonin (3 mg kg^{-1}) did not modify the number of choices of the arm leading to the large reward (9.85±0.10 vs 9.83±0.11; n=13; t=0.00, *NS*).

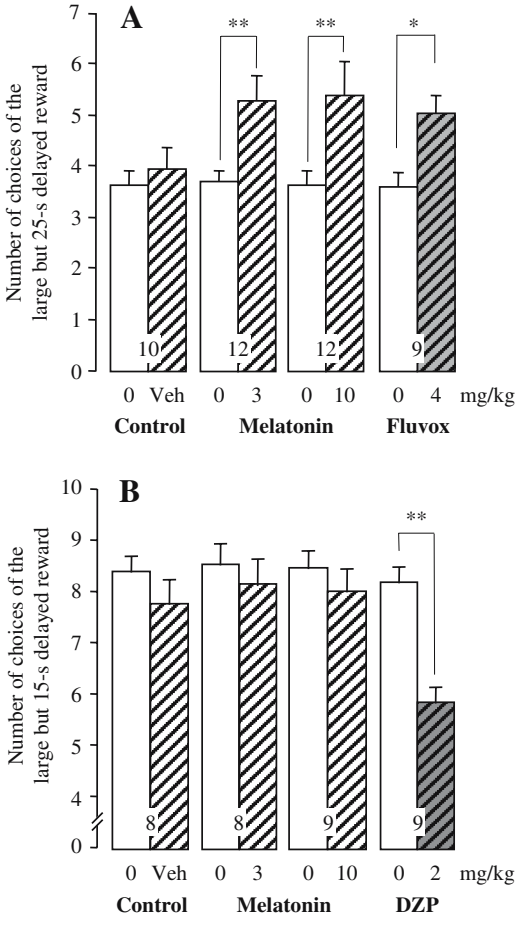

Fig. 1 Effects of melatonin, compared to fluvoxamine or diazepam, on the number of choices, in a T-maze, of the arm giving access to the large reward delayed by 25 s (**a**), or by 15 s (**b**). The *histograms* represent the mean (+SEM) total number of choices of the large-but-delayed reward during blocks of two successive sessions of five trials each (ten trials) after saline (baseline sessions, *white bars*) or drug/vehicle (drug sessions, *hatched bars*) injections. All rats received saline (0) i.p. before the baseline sessions. Melatonin (3 and 10 mg kg^{-1}), or its Tween vehicle (*Veh*) for the control group, was injected i.p., 30 min before the drug sessions. Fluvoxamine (*Fluvox*, 4 mg kg^{-1}), used as a positive control in the 25-s delay paradigm, and diazepam (*DZP*, 2 mg kg^{-1}), used as a positive control in the 15-s delay paradigm, were administered i.p., 30 min before the drug sessions. The number of rats per group is indicated in the histograms. *P<0.05; **P<0.01 drug vs baseline sessions (paired Student's t test)

Effect of agomelatine

The number of choices of the large-but-25 s-delayed reward was significantly increased with agomelatine at 10 mg kg^{-1} (t=3.79, P<0.02) and 30 mg kg^{-1} (t=3.35, P<0.01). The choice of control rats did not differ between baseline and vehicle sessions (t=0.20, NS) (Fig. 2).

Effect of S22153+melatonin

The choice of control (vehicle) animals and of rats given the $MT_{1/2}$-R antagonist S22153 (at a dose, 40 mg kg^{-1} i.p., previously shown to block the effects of $MT_{1/2}$-R agonists; Becker et al. 2004) was not modified as compared with baseline sessions (t=0.23 and 0.00, respectively, NS). As in the first experiment, melatonin (3 mg kg^{-1}) significantly increased the number of choices of the arm giving access to the large-but-25 s-delayed reward (t=3.74, P<0.01). Rats given S22153, 5 min before melatonin (3 mg kg^{-1}) also increased their choice of the large-but-delayed reward (t=3.00, P<0.05), indicating that S22153 did not counteract the effect of melatonin (Fig. 3).

Effect of GR205171

The choice of control rats did not differ between baseline and vehicle sessions (t=1.10, *NS*). GR205171, at the 30 mg kg^{-1}, but not the 10 mg kg^{-1} dose, increased the number of choices of the 25-s delayed reward (t=6.48, P<0.001 and t=0.66, NS, respectively). In the same series of experi-

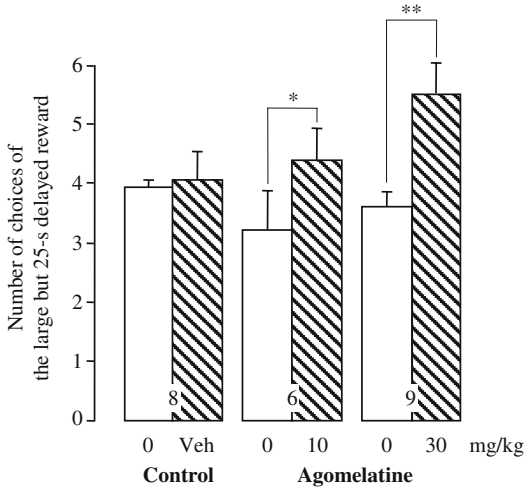

Fig. 2 Effects of agomelatine on the number of choices of the large but 25-s delayed reward in a T-maze. See legend to Fig. 1. Agomelatine (10 and 30 mg kg^{-1}) or its HEC vehicle (*Veh*) was injected i.p., 30 min before the drug sessions. *P<0.02; **P<0.01 drug vs baseline sessions (paired Student's t test)

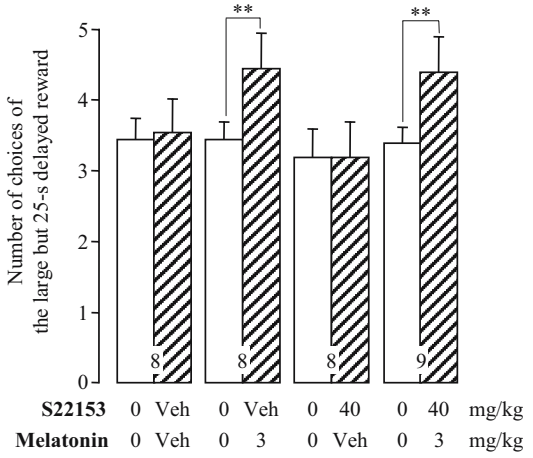

Fig. 3 Effects of the co-administration of a melatonin receptor antagonist (S22153) and melatonin on the number of choices of the arm giving access to the large but 25-s delayed reward in a T-maze. See legend to Fig. 1. Melatonin (3 mg kg^{-1}) or its vehicle was injected i.p., 30 min before the drug sessions. S22153 (40 mg kg^{-1}, i.p.) or its vehicle was injected 5 min before melatonin (or vehicle). **P<0.01 drug vs baseline sessions (paired Student's t test)

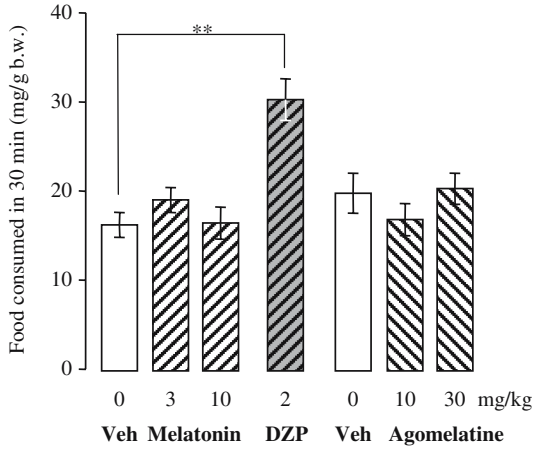

Fig. 5 Effects of melatonin and agomelatine on food intake—comparison with diazepam. The histograms represent the mean (±SEM) total quantity of food, expressed as mg g^{-1} of body weight, consumed in 30 min by rats food deprived for 16 h. Melatonin (3–10 mg kg^{-1}), agomelatine (10–30 mg kg^{-1}) and diazepam (*DZP*, 2 mg kg^{-1}), as positive control, or their respective vehicle (*Veh*= Tween or HEC in saline), were administered i.p., 30 min before the test. n=10 rats/group. **P<0.02 DZP vs. Tween vehicle (unpaired Student's t test)

ments, rats given the tricyclic antidepressant clomipramine (8 mg kg^{-1}) also increased their choice of the large-but-delayed reward (t=2.54, P<0.05) (Fig. 4).

Effect of melatonin and agomelatine on food intake

The quantity of food eaten by control rats was ca. 5.5 g in the group given Tween vehicle and 6.5 g in those given HEC vehicle. As depicted in Fig. 5, the quantity of food ingested during the 30-min test session was not significantly modified by melatonin (3–10 mg kg^{-1}) ($F_{2,27}$=1.03, NS) or agomelatine (10–30 mg kg^{-1}) ($F_{2,27}$=1.59, NS). In contrast, diazepam (2 mg kg^{-1}) significantly increased food intake (vs the Tween vehicle control group: t=2.75, P<0.02).

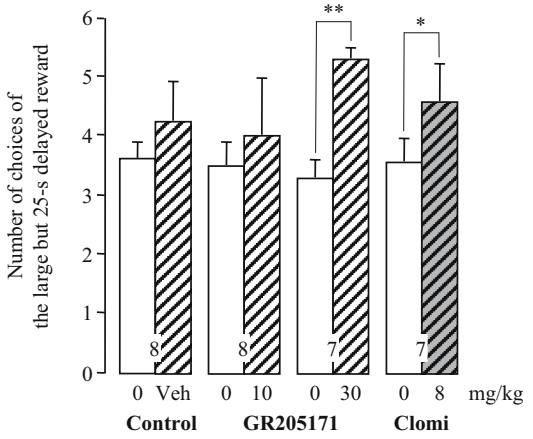

Fig. 4 Effects of GR205171, compared to clomipramine, on the number of choices of the arm giving access to the large but 25-s delayed reward in a T-maze. See legend to Fig. 1. GR205171 (10 and 30 mg kg^{-1}) or its vehicle was injected s.c., 30 min before the drug sessions. Clomipramine (*Clomi*, 8 mg kg^{-1}), used as a positive control, was injected i.p., 30 min before the drug sessions. *P<0.05; **P<0.01 drug vs baseline sessions (paired Student's t test)

Discussion

The present study showed that the NK_1-R antagonist, GR205171 (30 mg kg^{-1}), and the $MT_{1/2}$-R agonists, melatonin (3 and 10 mg kg^{-1}) and agomelatine (10 and 30 mg kg^{-1}), markedly affected the behaviour of rats under delay of reward conditions. Indeed, in a T-maze where animals were allowed to choose between a large reward (ten pellets) whose access (and consumption) was delayed by 25 s, and an immediate access to a smaller reward (two pellets), like the established ADs clomipramine (8 mg kg^{-1}) and fluvoxamine (4 mg kg^{-1}), these drugs increased the frequency of choice of the larger, delayed reinforcer. Thus, these compounds seem to enhance tolerance to delay of reward. Unexpectedly, however, the "anti-impulsivity" action of melatonin was not prevented by the $MT_{1/2}$-R antagonist, S22153, administered at a dose shown to reduce behavioural and biochemical effects of $MT_{1/2}$-R agonists in rats (Becker et al. 2004), suggesting that it was unlikely mediated by MT_1 and/or MT_2 receptors. An alternative primary target of melatonin could be the third low-affinity binding site (MT_3) described in brain and peripheral tissues of mammals as the quinone reductase 2 (QR_2) enzyme, whose pharmacological properties are distinct from those of MT_1 and MT_2 receptors (see, e.g. Nosjean et al. 2000). However, despite the availability of a QR_2 knock-out mouse, the exact in vivo role of this site remains to be established (Mailliet et al. 2004).

The melatonin-induced shift in choice behaviour was observed at a dose that did not alter rats' choice under no delay conditions. In addition, when the delay of reinforcement was only 15 s, the high frequency of choice of the larger reward was not reduced by this neurohormone, in contrast to that observed with 2 mg kg^{-1} of diazepam (present study; Thiébot et al. 1985a). This suggests that melatonin did not impair stimulus control (in cognitive terms, disruption of attention or memory) that could lead to a random selection of the arms of the T-maze. Agomelatine has been reported to reduce spontaneous locomotion in rats given the 30 mg kg^{-1} dose (Millan et al. 2005). However, the choice of rats given agomelatine can hardly be accounted for by such non-specific effects since the 10 mg kg^{-1} dose also increased the choice of the large-but-delayed reward without altering motor activity (Millan et al. 2005). On the other hand, since the reinforcers differed both in delay and size, one can argue that the treatments might have enhanced the relative value of the larger reward (irrespective of the delay), rather than attenuated the discounting value of the delay. However, such a possibility seems unlikely since, distinct from diazepam (2 mg kg^{-1}), melatonin and agomelatine did not increase the quantity of food consumed by moderately fasting rats, at doses active in the T-maze (25 s delay) procedure. Unfortunately, the available quantity of GR205171 was too small to allow similar control experiments for unveiling possible non-specific effects. However, in starving NK_1-R knock out mice, the latency to eat was not altered in home cage conditions (Santarelli et al. 2001), suggesting that these receptors play no major role in the salience of food value. In addition, since there was no evidence for sedation after the administration of another NK_1-R antagonist (L-760735), chemically related to GR205171, even at doses in excess of those required for central NK_1-R occupancy (Rupniak et al. 2001), it seems unlikely that the effect of GR205171 could be confounded by such an impairment. Therefore, the enhanced tolerance to delay induced by melatonin, agomelatine and GR205171 unlikely resulted from some non-specific effects. Interestingly, the magnitude of this action was similar to that observed with a variety of clinically active antidepressants, TCAs, MAOIs and SSRIs (present study; Bizot et al. 1988).

Serotoninergic neurones are claimed to play a crucial, though non-exclusive, role in impulse control. Indeed, numerous clinical studies showed that an inverse relationship exists between central 5-HT tone and impulsivity. In particular, low levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid have been regularly found in impulsive subjects who committed violent suicide attempts or aggressive acts (Linnoila et al. 1983; Virkkunen et al. 1995; Coccaro et al. 1998). Accordingly, drug- or neurotoxin-induced decreases in 5-HT function enhanced impulsive-like responding in various animal procedures, including the T-maze paradigm, whereas an increase in 5-HT neurotransmission seems to improve impulse control (Fletcher 1995; Harrison et al. 1997; Al-Ruwaitea et al. 1999; Bizot et al. 1999). Therefore, it can be hypothesized that the 5-HT system participates in the "anti-impulsivity" effect of melatonin, agomelatine and the NK_1-R antagonist GR205171. However, NK_1-R antagonists have been claimed to act independently of the 5-HT system (Kramer et al. 1998), and despite some data suggesting that a long-term impairment of NK_1-R-mediated functions may enhance 5-HT neurotransmission in forebrain structures, this does not apparently occur upon acute or sub-acute blockade of NK_1-R (Millan et al. 2001; Blier et al. 2004). Hence, in the present T-maze study, the increased tolerance to delay of gratification observed in rats given two injections of GR205171, 24 h apart, was unlikely accounted for by an enhanced 5-HT neurotransmission.

With regard to melatonin, some studies also suggested its interaction with the 5-HT system, but results published so far in the relevant literature are rather variable. On one hand, behavioural effects of melatonin (hypoactivity, grooming, sniffing) were prevented by bilateral microinjections of 5-HT and a variety of ADs into the nucleus accumbens (Gaffori and Van Ree 1985). On the other hand, fragmentary biochemical data obtained in awake rats indicated that high doses (60 mg kg^{-1}, i.p., and ca. 30 mg kg^{-1}, p.o.) of melatonin may modulate (enhance or reduce) 5-HT release within some cerebral structures (Chuang and Lin 1994; Yoshioka et al. 2000), whereas other studies, also with high doses, led to the conclusion that melatonin does not affect 5-HT release in main projection areas of 5-HT neurones such as frontal cortex and hippocampus (Millan et al. 2005). Using considerably lower doses, ex vivo studies indicated that melatonin (0.5 or 1 mg kg^{-1}, s.c.) increased tissue levels of 5-HT and 5-HIAA in most hypothalamic structures, in the amygdala and in the midbrain, but displayed an inhibitory effect on 5-HT turnover in suprachiasmatic nuclei (Miguez et al. 1994, 1996). Therefore, whether 5-HT neurotransmission actually participates in the behavioural effects of melatonin remains to be firmly established.

Taken together, these data suggest that, although a participation of the 5-HT system cannot be totally excluded, the effects of acute injections of GR205171 or melatonin were unlikely mediated through 5-HT processes only.

By contrast, agomelatine acts directly on 5-HT function. Indeed, this potent agonist at melatonin receptors is also an antagonist at $5\text{-HT}_{2B/2C}$ receptors, although its in vitro affinity for the latter two sites is ca. 1,000-fold lower than for MT_1- and MT_2-R (Millan et al. 2003, 2005) (see Table 1). Accordingly, the in vivo effects which depend on the interaction of agomelatine with $5\text{-HT}_{2B/2C}$-R occur in a range of doses higher than those active in chronobiotic paradigms responsive to $MT_{1/2}$-R stimulation (Van Reeth et al. 2001). However, the degree of dose separation (10- to 20-fold) is less pronounced than expected from such differential in vitro affinities. For instance, penile erections induced in rats by 5-HT_{2C}-R agonists were significantly antagonized by agomelatine in the 10–40 mg kg^{-1} dose range (Chagraoui et al. 2003; Millan et al. 2003).

According to Papp et al. (2003), the behavioural effects of agomelatine might involve different mechanisms depending on the time of its administration during the day. Indeed, agomelatine showed an antidepressant-like effect

in the chronic mild stress procedure whether it was administered in the morning or in the evening, but only the effect of evening treatment could be prevented by $MT_{1/2}$-R blockade by S22153. Accordingly the morning action of agomelatine was unlikely accounted for by an activation of $MT_{1/2}$-R, but might involve the blockade of 5-$HT_{2B/2C}$ receptors (Papp et al. 2003). Taking these data into consideration, it can be hypothesized that in the present study, agomelatine, which was injected in the morning and at high doses, could exert its "anti-impulsivity" effect preferentially via 5-$HT_{2B/2C}$-R. Recent studies using selective receptor ligands showed a complex involvement of the 5-HT_2 receptor subtypes in impulse-related behaviour. In particular, 5-HT_{2A}- and 5-HT_{2C}-R seem to have opposing roles in impulsive-related behaviour. Antagonising 5-HT_{2A}-R was reported to reduce premature responses—an index of impulsive behaviour—on a five-choice serial reaction time task (5-CSRT) (Winstanley et al. 2003, 2004). In contrast, consonant with animal and human studies suggesting a deleterious effect of a reduction of 5-HT function on impulse control, 5-HT_{2C}-R blockade increased premature responding on the 5-CSRT (Higgins et al. 2003; Winstanley et al. 2004). The release of response suppression in conflict procedures of anxiety by 5-HT_{2C}-R antagonists (Kennett et al. 1997) can also be related to such "pro-impulsive" action. Indeed, these two effects are shared by benzodiazepines, and a relationship has been tentatively established between release of behavioural suppression and reduction of tolerance to delay (Thiébot et al. 1985b). In this context, the 5-HT_{2C}-R antagonist properties of agomelatine very unlikely contributed to (but could even counteract) its "anti-impulsivity" effect observed in the T-maze.

On the other hand, although a role for 5-HT_{2B}-R in impulse control, if any (see Higgins et al. 2003), remains to be established, the stimulation of 5-HT_{2B}-R has been reported to induce behavioural suppression in conflict procedures (Kennett et al. 1998). Therefore, in line with the above hypothesis, the blockade of these receptors by agomelatine might be involved in the observed improved tolerance to delay of reward.

Some studies also suggested an interaction of melatonin with γ-aminobutyric acid (GABA) neurotransmission. In vitro studies reported a benzodiazepine-like melatonin-induced increase in [^{3}H]GABA or [^{3}H]muscimol binding to synaptosomal fractions from rat cerebral cortex (Coloma and Niles 1988), and blockade by flumazenil of the benzodiazepine binding site on $GABA_A$ receptors was found to prevent some anxiolytic-like effects of melatonin (see, e.g. Kopp et al. 1999a). However, because melatonin actually exerted behavioural effects opposite to those of diazepam in the present T-maze paradigm (Fig. 1; see also Thiébot et al. 1985a,b), it is very unlikely that some benzodiazepine-like action of the neurohormone contributed to its anti-impulsivity effect.

The noradrenergic (NA) system has also been involved in impulsive-related behaviour (Coccaro et al. 2003), and relevant studies indicate that both NK_1-R antagonists and agomelatine can interact with NA neurotransmission. While single intravenous injections of most NK_1-R antagonists did not modify the spontaneous firing rate of NA neurones in rats (Haddjeri and Blier 2000), acute administrations of GR205171 were found to elevate the dialysate concentrations of NA in the frontal cortex (10–40 mg kg^{-1}, i.p.) and increased the firing rate of NA neurones in the locus coeruleus (2–4 mg kg^{-1}, i.v.) (Millan et al. 2001). Likewise, at high doses, agomelatine enhanced extracellular levels of NA in the frontal cortex (20–80 mg kg^{-1}, i.p.) and increased the firing rate of NA neurones (4–16 mg kg^{-1}, i.v.), whereas melatonin (40 mg kg^{-1}, i.p. or 16 mg kg^{-1}, i.v.) was ineffective in both indices of NA function, which led Millan et al. (2003, 2005) to ascribe agomelatine effects to the blockade of brain 5-HT_{2C} receptors in vivo in rats (see above). Thus, NA processes might also participate in the effects of both GR205171 and agomelatine in the T-maze procedure; further investigations, however, are needed to directly assess this hypothesis.

Finally, it must be noted that NK_1-R antagonists and $MT_{1/2}$-R agonists are also endowed with anxiolytic properties. Indeed, these compounds are effective in validated models of anxiety in rodents (Kopp et al. 2000; Cheeta et al. 2001; Varty et al. 2002; Millan et al. 2005) and have a good clinical efficacy in the treatment of anxiety, as evaluated by the Hamilton Anxiety Scale, at least in patients with major depressive disorders (Kramer et al. 1998; Lôo et al. 2002a). However, since anxiolytic drugs seem to lessen impulse control (Thiébot et al. 1985a; Bizot et al. 1999), the present results are not consistent with such properties, but rather add an experimental argument in favour of an antidepressant-like profile of NK_1-R antagonists and $MT_{1/2}$-R agonists.

In conclusion, like established antidepressants, an NK_1-R antagonist, GR205171, and two $MT_{1/2}$-R agonists, melatonin and agomelatine, enhance the waiting capacities of rats, possibly reflecting a shared capacity to enhance self-control in impulsive subjects. However, further investigations are necessary to elucidate the underlying neurobiological mechanisms.

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