University of Groningen

Anxiolytic-like action of the antidepressant agomelatine (S 20098) after a social defeat requires the integrity of the SCN

Tuma, J.; Strubbe, J.H.; Mocaer, E.; Koolhaas, J.M.

Published in:
European Neuropsychopharmacology

DOI:
[10.1016/j.euroneuro.2005.02.004](https://doi.org/10.1016/j.euroneuro.2005.02.004)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2005

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Tuma, J., Strubbe, J. H., Mocaer, E., & Koolhaas, J. M. (2005). Anxiolytic-like action of the antidepressant agomelatine (S 20098) after a social defeat requires the integrity of the SCN. *European Neuropsychopharmacology*, 15(5), 545-555. <https://doi.org/10.1016/j.euroneuro.2005.02.004>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Anxiolytic-like action of the antidepressant agomelatine (S 20098) after a social defeat requires the integrity of the SCN

Jolanda Tuma^a, Jan H. Strubbe^a, Elisabeth Mocaër^b, Jaap M. Koolhaas^{a,*}

^aDepartment of Animal Physiology, University of Groningen, Kerklaan, 30, 9751 NN, Haren, The Netherlands

^bInstitut de Recherches Internationales Servier, France

Received 4 May 2004; received in revised form 1 February 2005; accepted 15 February 2005

Abstract

In rats, social defeat by an aggressive opponent induces a state of anxiety, shown by a decrease in time spent on active explorative behaviour, an increase in immobility, a clear decrease in frequency of all active behavioural parameters (enhanced passivity).

We tested the hypothesis whether acute or sub-chronic agomelatine would antagonize the negative consequences of a social defeat. As many chronobiological actions of melatonin and its receptor agonist agomelatine require the integrity of the suprachiasmatic nuclei (SCN), we examined whether the anxiolytic-like action of agomelatine 1 day after a social defeat is still present in SCN-lesioned rats.

Sub-chronic administration of agomelatine caused a clear reduction of the social defeat induced behavioural consequences. A single agomelatine injection prior to the post-defeat test was less effective and a single melatonin injection was hardly effective. SCN lesion did not affect the anxiety reaction after a social defeat.

Thus, sub-chronic agomelatine treatment or a single agomelatine injection reduced a state of anxiety and passivity caused by a social defeat. The defeat-induced behavioural changes do not depend on the SCN but agomelatine showed its anxiolytic action only in sham-lesioned animals, which indicates that the anxiolytic-like action of agomelatine requires the integrity of the SCN. Mechanisms sustaining this activity are discussed.

© 2005 Elsevier B.V. and ECNP. All rights reserved.

Keywords: Agomelatine (S 20098); Social defeat; Rat; Suprachiasmatic nucleus of the hypothalamus

1. Introduction

Social defeat by an aggressive male conspecific is a natural stressor which is known to induce a state of stress and anxiety as expressed by more immobility and less exploration (Koolhaas et al., 1995; Miczek et al., 1990; Koolhaas et al., 1997). Such a defeat is known to induce acute and strong effects on cardiovascular and neuro-endocrine activation, hyperthermia and behaviour (Miczek et al., 1990; Bohus et al., 1987; Tornatzky and Miczek, 1994), and longer lasting effects on behavioural and physiological parameters (Koolhaas et al., 1990; Miczek et al., 1990; Meerlo et al., 1996a,b). Social defeat also causes a variety of alterations in the daily rhythms, i.e., a

sharp subsequent reduction in the amplitude of the daily temperature and activity rhythm, which lasted for at least 4 days (Meerlo et al., 1996b; Meerlo et al., 1997, 2002). Such rhythm disturbances may be due to effects of stress on sub-oscillators that are known to exist in many tissues, which are normally under the control of the SCN, or due to other effects of stress that mask the output of the circadian system.

Agomelatine (S 20098) is a new antidepressant active in preclinical models (Bertaina-Anglade et al., 2002; Papp et al., 2003) and with demonstrated clinical efficacy in major depressive disorders (Lêo et al., 2002). The activity of agomelatine is independent of the time of administration and appears to involve a combination of its agonism at melatonin MT₁ and MT₂ (Yous et al., 1992; Ying et al., 1996; Conway et al., 2000) and antagonism at 5-HT_{2C} receptors (Cussac et al., 2002; Millan et al., 2003).

* Corresponding author. Tel.: +31 50 363 2338; fax: +31 50 363 2331.

E-mail address: j.m.koolhaas@biol.rug.nl (J.M. Koolhaas).

Agomelatine shows a high affinity for cloned human receptors MT₁ and MT₂ subtypes ($K_i=6.2 \times 10^{-11}$ M and 2.7×10^{-10} M respectively), which is comparable to that of melatonin, and also binds to serotonin 5-HT_{2C} subtype ($pK_i=6.15$). In line with its melatonin agonist property, agomelatine has similar chronobiological properties as melatonin (Lewy et al., 1996; Benloucif and Dubocovich, 1996; Redman et al., 1983; Illnerova et al., 1989; Golombek and Cardinali, 1993), and can resynchronize disrupted circadian rhythms (Armstrong et al., 1993; Redman et al., 1995; Martinet et al., 1996; Van Reeth et al., 1994; Weibel et al., 2000) as well as restore the phase shifting response to a dark pulse (Van Reeth et al., 2001). The re-entraining activity of free-running rhythms by agomelatine is dose-dependent, and shows a clear relationship to plasma concentration of agomelatine (Martinet et al., 1996). These chronobiological properties of agomelatine, like melatonin (Lewy et al., 1996; Benloucif and Dubocovich, 1996; Cassone et al., 1986), are phase-dependent and require the integrity of the suprachiasmatic nucleus of the hypothalamus (SCN) (Redman and Francis, 1998). Like melatonin (Margraf and Lynch, 1993b), agomelatine suppresses the firing rates of SCN cells in a dose-dependent manner (Ying et al., 1996). Several studies have shown that human depression is often associated with a disturbance in circadian rhythms, and raised the hypothesis of a role for melatonin and melatonin agonists in the regulation of mood and in mood disorders. Clinical studies suggest that treatment with melatonin reduces pre-operative anxiety (Naguib and Samarkandi, 2000) and studies in mice, rats report anxiolytic actions of melatonin (Guardiola-Lemaitre et al., 1992; Pierrefiche et al., 1993; Kopp et al., 1999, 2000).

Agomelatine is also an antagonist at the 5-HT_{2C} receptor ($IC_{50}=2.7 \times 10^{-7}$ M) and a number of studies indicate that compounds antagonizing this receptor subtype exhibit anxiolytic activity. Kennett et al. (1997) have shown an anxiolytic profile of the 5-HT_{2C} specific antagonist SB 242,084 in the rat social interaction test and Geller–Seifter conflict test of anxiety. Similar activity has been demonstrated with the use of less selective antagonists, in the elevated plus-maze test (Rocha et al., 1994; Griebel et al., 1997; Mora et al., 1997), the rat social interaction test (Kennett, 1992), the rat Geller–Seifter and conflict tests (Cervo and Samanin, 1995; Kennett et al., 1994, 1995, 1996; Griebel et al., 1997).

Collectively, given that social defeat causes a disorganization of internal rhythms in animals, and in view of the anxiolytic properties of melatonin and antagonists of 5-HT_{2C} receptors in the literature, one can hypothesize that the chronobiotic properties of agomelatine together with its 5-HT_{2C} receptors antagonist property could oppose the behavioural consequences of the social defeat.

The present study therefore explores the effects of acute or sub-chronic agomelatine on the initial anxiety behaviour obtained in the first few days after a social defeat. The

effects of acute agomelatine were compared to those of melatonin. Finally, the chronobiological properties of agomelatine suggest that the mood affecting properties of agomelatine requires the integrity of the SCN. To test this hypothesis, the acute effect of agomelatine was examined on anxiety behaviour of SCN-lesioned and sham-operated rats subjected to social defeat.

2. Material and methods

2.1. Animals and housing

The present experiments were performed with male Wistar rats ($n=133$, 3–4 months of age at the beginning of the experiment) individually housed in cages ($30 \times 40 \times 20$ cm) with free access to food and water throughout the experiment. The animals were housed under light–dark conditions LD: 12:12h, with dark from 12.00 to 24.00 h. All manipulations were performed during the first half of the dark phase.

Food intake was recorded throughout the light–dark cycle, using a food-weighing system. The movements and the weight of the food hopper were recorded in bouts of 6 min and used as an indicator of the animals' rhythm.

2.2. Social conflict

All animals were exposed to a social defeat or a control procedure. In the “acute” experiment, all animals were exposed to first a control procedure and then a social defeat with 1 week in-between, i.e., a within subject design. In the “sub-chronic” experiment, a between-subject design was used; the animals were exposed to either the control procedure or the social defeat.

2.2.1. Social defeat

The social defeat paradigm was performed as already described (Meerlo et al., 1996a). Briefly, a male Wistar rat was placed in the home cage of a dominant wild-type rat. This resident was housed in a large home cage together with a female rat, to facilitate territorial behaviour. Before the experimental Wistar rat was introduced into the home cage of the resident, the female rat was removed. Generally, when the Wistar male rat was introduced in the cage, the resident investigated and attacked the intruder within 2 min. When the intruder showed clear submissive behaviour, e.g., lying on the back, the intruder was confined to a small wire mesh cage in the middle of the home cage during the remaining hour. Then the rat was returned to its own cage and brought back to the animal room.

2.2.2. Control procedure

A male Wistar rat was placed in a clean cage in a separate test room during 1 h, then was returned to its own cage and brought back to the animal room.

2.2.3. Post-defeat test

One day after the social defeat or the control procedure, the animals were subjected to a post-defeat test. Each experimental animal was brought again to the test room where the dominant rats were housed. The female rat was removed from the dominant cage, and the resident male itself was enclosed in a small wire mesh cage and placed in the middle of its territory. Subsequently, the experimental animal was introduced into the cage of the dominant rat from which it had lost the fight 1 day before, or, in the case of the non-defeated controls, an unknown dominant. During 10 min, the behaviour of the experimental animal was recorded on video tape. Using a standard ethogram, both the percentage time and the frequency of behavioural parameters were used for analysis.

2.3. Surgery

Animals received either an electrolytic lesion ($n=26$) in the region of the suprachiasmatic nucleus or were sham lesioned ($n=38$) under halothane anaesthesia. Because of the elongated shape of the SCN, double bilateral lesions (2 m Amp, 10 s) were placed at the following co-ordinates: 0.9 mm posterior and 9.1 mm ventral to Bregma, 0.3 mm left and right from midline; 1.4 mm posterior and 9.1 mm ventral to Bregma, 0.3 mm left and right from midline.

For sham lesions, the electrode was inserted into the brain like during the lesion procedure, while no current was given.

After surgery, feeding rhythmicity was used to examine the success of the SCN lesion. The criterion for the success of the SCN lesion was that the SCN were totally destructed, while the rhythm was totally disturbed. At the end of the experiment, locations of the lesions were checked by histological examination on brain tissue sections.

2.4. Treatments

2.4.1. Acute effects of agomelatine and melatonin (experiment 1)

Five minutes prior to the post-defeat test, the animals were injected intraperitoneally with vehicle (cyclodextrin 40%) ($n=8$), 5 mg/kg agomelatine ($n=8$), 10 mg/kg agomelatine ($n=8$), 5 mg/kg melatonin ($n=8$) or 10 mg/kg melatonin ($n=8$) in cyclodextrin solution. The solutions were freshly made maximally 4 h before the test.

2.4.2. Sub-chronic treatment with agomelatine (experiment 2)

Three days prior to the social defeat or the control procedure, standard food pellets were replaced by food pellets containing 750 parts per million (ppm) agomelatine ($n=6$ social defeat and $n=6$ non-defeat control), 1500 ppm agomelatine ($n=5$ for social defeat and $n=6$ for non-defeat control) or control food ($n=6$ for both social defeat and control procedure).

2.4.3. Effects of agomelatine in sham- or SCN-lesioned rats

The animals were assigned to one of the following groups:

- Sham lesioned, vehicle control (40% cyclodextrin)
- Sham lesioned, agomelatine treatment (5mg/kg dissolved in 40% cyclodextrin)
- SCN lesioned, vehicle control (40% cyclodextrin)
- SCN lesioned, agomelatine treatment (5mg/kg dissolved in 40% cyclodextrin)

Vehicle control or agomelatine was administered via i.p. injection, 10 min prior to the post-defeat test.

After visual verification of the locations of the lesion, combined with the visual assessment of the feeding rhythms the number of animals in the non-defeated control groups were (a) $n=10$; (b) $n=9$; (c) $n=5$; (d) $n=8$, and in the social defeat groups (a) $n=9$; (b) $n=10$; (c) $n=7$; (d) $n=6$.

2.5. Statistics

To test the overall effect of social defeat and pharmacological treatment on the behavioural parameters, a two-way ANOVA was performed with defeat condition and treatment as independent variables.

To test the effect of social defeat in itself on the percentage time and frequency of the behavioural parameters, a paired *t*-test (acute administration) or an independent samples *t*-test (sub-chronic treatment) was performed for the groups that did not receive pharmacological treatment as paired or independent variables.

To test the effect of pharmacological treatment after social defeat on the percentage time and frequency of the behavioural parameters, a Kruskal Wallis one-way ANOVA was used, with treatment groups as independent variables.

Two-way ANOVA was performed to test the overall effect of SCN lesion, agomelatine treatment and the interaction (SCN lesion $\times$ agomelatine treatment) after a social defeat and after the control procedure. Mann Whitney *U* tests were performed to test the effect of a social defeat in sham-lesioned non-treated and SCN-lesioned non-treated groups, the effect of SCN lesion within non-defeated non-treated groups, socially defeated, non-treated, non-defeated agomelatine treated and socially defeated agomelatine treated groups, and the effect of agomelatine treatment within sham-lesioned non-defeated, sham-lesioned socially defeated, SCN-lesioned non-defeated and SCN-lesioned socially defeated groups.

3. Results

3.1. Social defeat

Two-way ANOVA revealed an overall effect of defeat on the majority of the behavioural parameters. Defeated

animals showed less exploration, increased immobility and increased investigation of the enclosed aggressive dominant. The effects of a social defeat were very consistent, as shown by the highly comparable results from acute and sub-chronic experiments.

Fig. 1 shows the main effects of social defeat as measured in the groups that did not receive pharmacological treatment (vehicle controls). A social defeat caused a significant decrease in time spent on exploring behaviour (i.e., walking and investigating the dominant home cage) and a significant increase in time spent on sniffing the enclosed aggressive dominant. In the sub-chronic experiment, social defeat caused a significant increase in the percentage time spent on immobility and a significant decrease in time spent rearing.

Another striking effect of a social defeat was the decrease in the frequency of several behavioural parameters after a social defeat compared to the non-defeated control. In the acute experiment, the total number of switches from one behaviour to another (total frequency) was significantly decreased after a social defeat compared to the control procedure ($p < 0.05$) (Fig. 2). This decrease in total frequency was mainly caused by a significant decrease in frequency of the active components walking and investigating the home cage, while the mean time per bout for these behaviours was not changed. On the other hand, the frequency of the more passive components immobility and sniffing the enclosed dominant was unchanged while the mean time per bout of these behaviours was significantly increased after a social defeat (Fig. 3).

In the sub-chronic experiment, the decrease in the overall number of behavioural switches in defeated animals was

Fig. 1. Effect of social defeat on percentage time spent on exploring, immobility and sniffing the enclosed dominant. * $P < 0.05$; paired t -test. ## $P < 0.01$; t -test.

Fig. 2. Effect of social defeat on frequency of all measured behavioural parameters in total. * $P < 0.05$; paired t -test.

close to significance ($p < 0.05$) (Fig. 2). Again, as in the acute experiment, the decrease in total frequency was the result of a significant decrease in frequency of the active components exploration and rearing. The time per bout of these behaviours was again unchanged (exploration) or even decreased (rearing). On the other hand, the frequency of immobility, investigating the enclosed aggressive dominant and stretching was unchanged or increased (immobility), while the mean time per bout of these more passive behaviours was increased (Fig. 4).

Fig. 3. Effect of social defeat on frequency and mean time per bout of the parameters walking, investigation of the home cage, immobility and sniffing the enclosed dominant. Results from acute experiment. * $P < 0.05$; paired t -test.

Fig. 4. Effect of social defeat on frequency and mean time per bout of the parameters exploration, rearing, immobility and sniffing the enclosed dominant. Results from experiment 2. ## $P < 0.05$; ### $P < 0.01$; t -test.

3.2. Acute effect of agomelatine and melatonin

In the social defeated animals, the percentage time walking was dose-dependently increased in agomelatine treated animals ($p < 0.05$), while immobility behaviour was decreased, although not significantly. The percentage time exploring the enclosed aggressive dominant (sniffing dominant) was not affected by agomelatine treatment. Melatonin did not significantly affect the behaviour after a social defeat. However, the percentage time immobility shows a trend towards a decrease in the melatonin treated

groups (Fig. 5). Neither agomelatine nor melatonin did affect the behaviour of the experimental rat after the control procedure.

The decrease in frequency of all behavioural switches in the defeated animals was normalised by agomelatine treatment in a dose-dependent manner ($p < 0.05$) (Fig. 6). The increase in total frequency was caused by a significant dose-dependent increase in frequency walking ($p < 0.05$) while the mean time per bout walking was unchanged. The frequency of investigation of the enclosed aggressive dominant was also increased ($p < 0.01$), but the mean time per bout of this component was decreased ($p < 0.05$). Melatonin did not affect the frequency of the switches from one to another behavioural component after a social defeat. In the non-defeated control animals neither agomelatine nor melatonin did affect the total frequency.

3.3. Effect of sub-chronic agomelatine

Sub-chronic agomelatine treatment caused a dose-dependent increase of the time spent on the active components exploring ($p < 0.01$) and rearing ($p = 0.001$) and a dose-dependent decrease of the time spent on investigating the enclosed aggressive dominant ($p < 0.05$) in the experimental rat after a social defeat. Sub-chronic agomelatine treatment caused a non-significant trend towards less immobility (Fig. 7). In the non-defeated control animals, sub-chronic agomelatine treatment had no effect.

After social defeat, the frequency of all measured behavioural parameters in total was, although not significantly, decreased. Sub-chronic agomelatine treatment caused a dose-dependent increase in this total frequency ($p < 0.05$) (Fig. 8). This increase in total frequency was

Fig. 5. Acute effect of a single agomelatine (S 20098) or melatonin injection on percentage time walking, immobility and sniffing the enclosed dominant after a social defeat. * $P < 0.05$ Kruskal Wallis one-way ANOVA.

Fig. 6. Acute effect of a single agomelatine (S 20098) or melatonin injection on total frequency of all behavioural parameters measured, after a control procedure and after a social defeat. White bar—control; grey bar—5 mg/kg agomelatine/melatonin; black bar—10 mg/kg agomelatine/melatonin. * $P < 0.05$; paired t -test, # $P < 0.05$; Kruskal Wallis one-way ANOVA.

caused by a significant dose-dependent increase in frequency exploring ($p < 0.01$) and rearing ($p < 0.01$). The mean time per bout of exploring is unchanged, while the mean time per bout of rearing was even increased ($p < 0.01$).

3.4. Social defeat and SCN lesion

SCN lesions neither affected the behaviour after social defeat nor after the control procedure. There were no significant differences between SCN-lesioned and sham-lesioned animals, and the impact of social defeat was similar

for SCN-lesioned and sham-operated animals. Fig. 9 shows the percentage time spent on walking, rearing, immobility and investigating the enclosed dominant as measured in the experimental rats 1 day after a social defeat or its control procedure. Two-way ANOVA revealed a significant effect of condition (social defeat or control procedure) on walking ($F_{1,62} = 94.346$; $p < 0.001$), immobility ($F_{1,62} = 21.944$; $p < 0.001$), sniffing the cage ($F_{1,62} = 46.688$; $p < 0.001$) and investigating the enclosed dominant ($F_{1,62} = 25.064$; $p < 0.001$). The percentage time walking and rearing was significantly decreased in both sham-lesioned ($p < 0.001$ and $p < 0.001$ respectively) and SCN-lesioned animals ($p < 0.001$ and $p < 0.01$, respectively) after a social defeat

Fig. 7. Effect of sub-chronic agomelatine (S 20098) treatment on percentage time spent on exploring, rearing, immobility and sniffing the enclosed dominant. # $P < 0.05$; ## $P < 0.01$; Kruskal Wallis one-way ANOVA.

Fig. 8. Effect of sub-chronic agomelatine (S 20098) treatment on total frequency of all behavioural parameters measured, after a control procedure and after a social defeat. # $P < 0.05$; Kruskal Wallis one-way ANOVA.

Fig. 9. Effect of social defeat on walking, rearing, immobility and investigation of the enclosed dominant. *—Significant difference between social defeat and its control procedure within sham-operated and SCN-lesioned (SCNX) groups ($p < 0.05$, Mann-Whitney U test).

compared to a control procedure. The percentage time immobility was significantly increased in both sham-lesioned ($p < 0.05$) and SCN-lesioned animals ($p < 0.05$) after social defeat compared to the control procedure. The percentage time investigating the enclosed dominant is significantly increased in SCN-lesioned animals ($p < 0.01$) after a social defeat compared to a control procedure. In the sham-lesioned animals, this did not reveal statistical significance.

3.5. Social defeat, SCN lesion and agomelatine treatment

In the non-defeated control animals, agomelatine treatment had no effect on any of the behavioural parameters, neither in sham-operated nor in SCN-lesioned animals

(Fig. 10). The experimental animal showed mainly walking, sniffing the cage, rearing and investigation of the enclosed dominant.

In the defeated animals, agomelatine did have a clear effect but only in the sham-operated animals. Two-way ANOVA revealed a significant interaction effect (SCN lesion $\times$ agomelatine treatment) on walking ($F_{1,62} = 4.257$; $p < 0.05$). In sham-operated socially defeated animals, agomelatine treatment caused a significant increase in percentage time walking ($p < 0.05$), and a significant decrease in percentage time immobility ($p = 0.01$). In SCN-lesioned socially defeated animals, agomelatine treatment did not affect any of the behavioural parameters. Fig. 11 shows the percentage time walking, rearing, immobility and investigation of the enclosed dominant

Fig. 10. Effect of agomelatine (S 20098) treatment on walking, rearing, immobility and investigation of the enclosed dominant, as measured 1 day after a control procedure in both sham- and SCN-lesioned (SCNX) animals.

Fig. 11. Effect of agomelatine (S 20098) treatment on walking, rearing, immobility and investigation of the enclosed dominant, as measured 1 day after a social defeat in both sham- and SCN-lesioned (SCNX) animals. *—Significant difference between non-treated and agomelatine-treated sham-operated animals ($p < 0.05$; Mann Whitney U test).

as measured 1 day after a social defeat in both sham-operated and SCN-lesioned animals.

4. Discussion

Social defeat caused a clear reduction in behavioural activity, an increase in immobility and an increase in exploring the enclosed aggressive dominant, as measured 1 day after defeat. These social defeat-induced changes are clearly diminished by sub-chronic administration of agomelatine and to a lesser extent by its acute intraperitoneal administration. By contrast, the acute melatonin did not significantly affect the social defeat-induced behavioural changes.

The action of agomelatine in socially defeated animals as shown in sham-operated animals is not found in SCN-lesioned animals. According to the suggestion that social defeat induces a state of stress and anxiety (Koolhaas et al., 1995; Miczek et al., 1990; Koolhaas et al., 1997), the present study shows that agomelatine might have an anxiolytic or anti-stress action that requires the integrity of the SCN.

The defeat-induced decrease in frequency of all behavioural parameters together is an indication of an enhanced passivity, since this decrease is the result of a decrease in frequency of active behavioural components while the mean time per bout of these active components was unchanged. On the other hand, the frequency of more passive behavioural components was unchanged, while the mean time per bout of these components was increased. This increased passivity is consistent with behavioural changes after defeat observed in open field tests (Meerlo et al., 1996a).

The social defeat-induced behavioural changes were clearly diminished by sub-chronic agomelatine treatment

and to a lesser extent by a single agomelatine injection, as shown by an increase in time spent on active behaviour and a trend towards less time spent on more passive behaviour. The effect of agomelatine is even more clearly demonstrated by the increase in total frequency. Both the acute administration of agomelatine 5 min prior to the post-defeat test and the sub-chronic treatment 3 days prior to the social defeat, are effective. This indicates that the exposure to agomelatine during the post-defeat test is the most important factor in the anxiolytic/anti-stress action. Moreover, since agomelatine affects the behaviour of the experimental animal after a social defeat only and not after the control procedure, one may suggest that the effect of agomelatine is state dependent.

Melatonin was far less effective in the post-defeat test compared to agomelatine, although the effects of both melatonin and agomelatine were in the same direction towards more active behaviour. This difference in effectiveness might be related to a longer lasting action of agomelatine on neuron activity than melatonin itself (Ying et al., 1996).

The mechanism behind the anxiolytic-like action of agomelatine is unknown, although several possible mechanisms can be suggested. The observed effects of agomelatine may be due to a specific and indirect effects on other behavioural and physiological mechanisms. However, experiments measuring body temperature and activity using radiotelemetry did not show any major effects of agomelatine, nor did we observe any effects on body weight. The most robust option might be that agomelatine acts via melatonin receptors, since it is a selective melatonin receptor agonist (Bonnefond et al., 1993; Dubocovich et al., 1993). One of the primary brain sites, which might be involved in this anxiolytic action, is the SCN. Agomelatine treatment shows similar effects in sham-operated socially

defeated animals as in un-operated animals. However, the anxiolytic-like action of agomelatine in socially defeated animals as shown in sham-operated animals is not found in SCN-lesioned animals. The SCN lesion in itself did not affect the behaviour, neither in defeated nor in non-defeated controls. Therefore, it seems that the defeat-induced behavioural changes and anxiety do not require an intact SCN. This is in line with findings of Meerlo et al. (1997), who showed that defeat induced changes in activity and body temperature rhythm are not caused by direct alterations of clock function.

The fact that the anxiolytic action of agomelatine is absent in SCN-lesioned animals suggests a direct action of agomelatine within the SCN, may be through the high density of melatonin receptors located in this nucleus. Moreover, agomelatine inhibits SCN neuronal firing (Ying et al., 1996), and one may speculate that this neuronal inhibition affects the CRH secretion and consequently the behavioural response to stress. Another alternative concerns the serotonergic projection to the SCN and intergeniculate leaflet from the raphe nucleus (Moore, 1973; Meyer-Bernstein and Morin, 1996). The raphe nuclei seem to be involved in activity-induced effects on SCN function (Kawano et al., 1996). Moreover, the dorsal raphe nucleus contains a high density of 5-HT_{1A} receptors, which seem to play a crucial role in the physiological and behavioural adaptation to stress and anxiety (Deakin, 1991). Therefore, the anxiolytic-like action of agomelatine might act indirectly via the 5-HT_{1A} receptor located in the dorsal raphe. However, this hypothesis is not supported by recent data showing the inability of repeated administration of agomelatine to desensitise 5-HT_{1A} autoreceptor in the dorsal raphe as well as to affect the electrophysiological functioning of postsynaptic 5-HT_{1A} receptors (Hanoun et al., 2004).

The SCN also contains a high density of 5HT_{2C} receptors (Rocha et al., 1994), and agomelatine, as a 5HT_{2C} antagonist (Millan et al., 2003), may act directly via these receptors. Actually, antagonists at this receptor subtype exhibit anxiolytic-like properties in different animal models (Rocha et al., 1994; Griebel et al., 1997; Mora et al., 1997; Kennett, 1992; Cervo and Samanin, 1995; Kennett et al., 1994, 1995, 1996, 1997). However, sub-chronic agomelatine doses used here (750–1500 ppm) resulted in peak plasma concentrations ranging from 11–25 ng/ml and 18–35 ng/ml, respectively (Tuma et al., 2001), corresponding to acute oral administration of 2.5 to 5 mg/kg (Martinet et al., 1996). Thus, with regard to these relatively low doses, together with the low affinity of agomelatine for 5HT receptors relative to the affinity for melatonin receptors, participation of 5-HT receptors in the anxiolytic-like action of agomelatine may be only minor.

Another mechanism by which melatonin and agomelatine might act is the GABA-benzodiazepine receptor system since several studies have shown that the analgesic, anxiolytic, anticonvulsant and sedative effect

of melatonin can be blocked by a benzodiazepine antagonist (Pierrefiche et al., 1993; Golombek et al., 1991a,b). However, binding studies have revealed no affinity of agomelatine for benzodiazepine receptor sites and rats receiving agomelatine do not discriminate for diazepam (Wiley et al., 1998). Thus, if any relationship with the GABAergic system, only indirect action of agomelatine should be hypothesized.

This study demonstrates that state of anxiety and passivity caused by a social defeat can be clearly reduced by a single agomelatine injection or a sub-chronic agomelatine treatment. The defeat-induced behavioural changes do not depend on the SCN but the anxiolytic-like action of agomelatine requires the integrity of the SCN. Finally, the anxiolytic activity reported here makes agomelatine a promising candidate in treating anxiety symptoms associated with depression.

References

- Armstrong, S.M., McNulty, O.M., Guardiola-Lemaitre, B., Redman, J.R., 1993. Successful use of S20098 and melatonin in an animal model of delayed sleep-phase syndrome (DSPS). *Pharmacol. Biochem. Behav.* 46, 45–49.
- Benloucif, S., Dubocovich, M.L., 1996. Melatonin and light induce phase shifts of circadian activity rhythms in the C3H/HeN Mouse. *J. Biol. Rhythms* 11, 113–125.
- Bertaina-Anglade, V., Mocaër, E., Drieu la Rochelle, C., 2002. Anti-depressant-like action of S 20098 (agomelatine) in the learned helplessness test. *Int. J. Neuropsychopharmacol.* 5, S65.
- Bohus, B., Benus, R.F., Fokkema, D.S., Koolhaas, J.M., Nyakas, C., Van Oortmersen, G.A., Prins, A.J.A., De Ruiter, A.J.H., Scheurink, A.J.W., Steffens, A.B., 1987. Neuroendocrine states and behavioral and physiological stress responses. In: De Kloet, E.R., Wiegant, V.M., De Wied, D. (Eds.), *Progress in Brain Research: Neuropeptides and Brain Function*. Elsevier, Amsterdam, pp. 57–70.
- Bonnefond, P., Martinet, L., Lesieur, D., Adam, G., Guardiola-Lemaitre, B., 1993. Characterization of S 20098, a new melatonin analog. In: Touitou, Y., Arendt, J., Pevet, P. (Eds.), *Melatonin and the Pineal Gland. From Basic to Clinical Application*. Excerpta Medica, Amsterdam, p. 123.
- Cassone, V.M., Chesworth, M.J., Armstrong, S.M., 1986. Entrainment of rat circadian rhythms by daily injection of melatonin depends upon the hypothalamic suprachiasmatic nuclei. *Physiol. Behav.* 36, 1111–1121.
- Cervo, L., Samanin, R., 1995. 5-HT(1A) receptor full and partial agonists and 5-HT(2C) (but not 5-HT3) receptor antagonists increases rates of punished responding in rats. *Pharmacol. Biochem. Behav.* 52, 671–676.
- Conway, S., Canning, S.J., Edward Howell, H.H., Mowat, E.S., Barrett, P., Drew, J.E., Delagrang, P., Lesieur, D., Morgan, P.J., 2000. Characterizations of human mt1/MT₂ receptors by CRE-luciferase reporter assay. *Eur. J. Pharmacol.* 390, 15–24.
- Cussac, D., Newman-Tancredi, A., Pasteau, V., Millan, M.J., 2002. Antagonist properties of the melatonin agonist S 20098 (agomelatine) at recombinant human (h) serotonin (5-HT)_{2C} receptors. *Int. J. Neuropsychopharmacol.* 5, S68.
- Deakin, J.F., 1991. Depression and 5HT. *Int. Clin. Psychopharmacol.* 3, 23–31.
- Dubocovich, M.L., North, P.C., Oakley, N.R., Hagan, R.M., 1993. A potent naphthalenic melatonin receptor agonist. In: Touitou, Y., Arendt, J., Pevet, P. (Eds.), *Melatonin and the Pineal Gland. From Basic to Clinical Application*. Excerpta Medica, Amsterdam, p. 123.

- Golombek, D.A., Cardinali, D.P., 1993. Melatonin accelerates reentrainment after phase advance of the light–dark cycle in Syrian hamsters: antagonism by flumazenil. *Chronobiol. Int.* 10, 435–441.
- Golombek, D.A., Escobar, E., Cardinali, D.P., 1991a. Melatonin-induced depression of locomotor activity in hamsters: time-dependency and inhibition by the central-type benzodiazepine antagonist Ro, 15-1788. *Physiol. Behav.* 49, 1091–1097.
- Golombek, D.A., Escobar, E., Burin, L.J., De Brito Sanchez, M.G., Cardinali, D.P., 1991b. Time-dependent melatonin analgesia in mice: inhibition by opiate or benzodiazepine antagonism. *Eur. J. Pharmacol.* 194, 25–30.
- Griebel, G., Perrault, G., Sanger, D.J., 1997. A comparative study of the effects of selective and non-selective 5-HT₂ receptor subtype antagonists in rat and mouse models of anxiety. *Neuropharmacology* 36, 793–802.
- Guardiola-Lemaitre, B., Lenegre, A., Porsolt, R.D., 1992. Combined effects of diazepam and melatonin in two tests for anxiolytic activity in the mouse. *Pharmacol. Biochem. Behav.* 41, 405.
- Hanoun, N., Mocaër, E., Boyer, P.A., Hamon, M., Lanfumey, L., 2004. Differential effects of the novel antidepressant agomelatine (S 20098) versus fluoxetine on 5-HT_{1A} receptors in the rat brain. *Neuropharmacology* 47, 515–526.
- Illnerova, H., Trentini, G.P., Maslova, L., 1989. Melatonin accelerates reentrainment of the circadian rhythm of its own production after an 8-h advance of the light–dark cycle. *J. Comp. Physiol.* 166, 97–102.
- Kawano, H., Decker, K., Reuss, S., 1996. Is there a direct retina–raphe–suprachiasmatic nucleus pathway in the rat? *Neurosci. Lett.* 212, 143–146.
- Kennett, G.A., 1992. 5-HT_{1C} receptor antagonists have anxiolytic-like actions in the rat social interaction model. *Psychopharmacology* 107, 379–384.
- Kennett, G.A., Pittaway, K., Blackburn, T.P., 1994. Evidence that 5-HT_{2C} receptor antagonists are anxiolytic in the rat Geller–Seifter model of anxiety. *Psychopharmacology* 114, 90–96.
- Kennett, G.A., Bailey, F., Piper, D.C., Blackburn, T.P., 1995. Effect of SB 200,646A, a 5-HT_{2C}/5-HT_{2B} receptor antagonist, in two conflict models of anxiety. *Psychopharmacology* 118, 178–182.
- Kennett, G.A., Wood, M.D., Bright, F., Cilia, J., Piper, D.C., Gager, T., Thomas, D., Baxter, G.S., Forbes, I.T., Ham, P., Blackburn, T.P., 1996. In vivo and in vitro profile of SB 206,553, a potent 5-HT_{2C}/5-HT_{2B} receptor antagonist with anxiolytic-like properties. *Br. J. Pharmacol.* 117, 427–434.
- Kennett, G.A., Wood, M.D., Bright, F., Trail, B., Riley, G., Holland, V., Avenell, K.Y., Stean, T., Upton, N., Bromidge, S., Forbes, I.T., Brown, A.M., Middlemiss, D.N., Blackburn, T.P., 1997. SB 242,084, a selective and brain penetrant 5-HT_{2C} receptor antagonist. *Neuropharmacology* 36, 609–620.
- Koolhaas, J.M., Hermann, P.M., Kemperman, C., Bohus, B., Van den Hoofdakker, R.H., Beersma, D.G.M., 1990. Single social defeat in male rats induces a gradual but long lasting behavioral change: a model of depression? *Neurosci. Res. Commun.* 7, 36–42.
- Koolhaas, J.M., Meerlo, P., Boer, S.F., Strubbe, J.H., Bohus, B., 1995. Social stress in rats: an animal model of depression? *Acta Neuropsychiatr.* 7, 27–29.
- Koolhaas, J.M., Meerlo, P., Boer, S.F., Strubbe, J.H., Bohus, B., 1997. The temporal dynamics of the stress response. *Neurosci. Biobehav. Rev.* 22, 1–8.
- Kopp, C., Vogel, E., Rettori, M.C., Delagrange, P., Guardiola-Lemaitre, B., Misslin, R., 1999. Effects of melatonin on neophobic responses in different strains of mice. *Pharmacol. Biochem. Behav.* 63, 521–526.
- Kopp, C., Vogel, E., Rettori, M., Delagrange, P., Misslin, R., 2000. Anxiolytic-like properties of melatonin receptor agonists in mice: involvement of mt1 and/or MT2 receptors in the regulation of emotional responsiveness. *Neuropharmacology* 39, 1865–1871.
- Lewy, A.J., Ahmed, S., Sack, R.L., 1996. Phase shifting the human circadian clock using melatonin. *Behav. Brain Res.* 73, 131–134.
- Lôo, H., Hale, A., D'Haenen, H., 2002. Determination of the dose of agomelatine, a melatonergic 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.
- Margraf, R.R., Lynch, G.R., 1993. Melatonin injections affect circadian behavior and SCN neurophysiology in Djungarian hamsters. *Am. J. Physiol.* 264, R615–R621.
- Martinet, L., Guardiola-Lemaitre, B., Mocaër, E., 1996. Entrainment of circadian rhythms by S20098 a melatonin agonist is dose and plasma concentration dependent. *Pharmacol. Biochem. Behav.* 54, 713–718.
- Meerlo, P., Overkamp, G.J.F., Daan, S., Hoofdakker, R.H., Koolhaas, J.M., 1996a. Changes in behaviour and body weight following a single or double social defeat in rats. *Stress* 1, 21–32.
- Meerlo, P., Boer, S.F.D., Koolhaas, J.M., Daan, S., Hoofdakker, R.H., 1996b. Changes in daily rhythms of body temperature and activity after a single social defeat. *Physiol. Behav.* 59, 735–739.
- Meerlo, P., Hoofdakker, R.H., Koolhaas, J.M., Daan, S., 1997. Stress-induced changes in circadian rhythms of body temperature and activity in rats are not caused by pacemaker changes. *J. Biol. Rhythms* 12, 80–92.
- Meerlo, P., Sgoifo, A., Turek, F.W., 2002. The effects of social defeat and other stressors on the expression of circadian rhythms. *Stress* 5, 15–22.
- Meyer-Bernstein, E.L., Morin, L.P., 1996. Differential serotonergic innervation of the suprachiasmatic nucleus and the intergeniculate leaflet and its role in circadian rhythm modulation. *J. Neurosci.* 16, 2097–2111.
- Miczek, K.A., Thompson, M.L., Tornatzky, W., 1990. Short and long term physiological and neurochemical adaptations to social conflict. In: Puglisi-Allegra, S., Oliverio, A. (Eds.), *Behavioural and Social Sciences*. Kluwer, Dordrecht, pp. 15–30.
- Millan, M.J., Gobert, A., Lejeune, F., Dekeyne, A., Newman-Tancredi, A., Pasteau, V., Rivet, J.M., Cussac, D., 2003. The novel melatonin agonist, agomelatine (S 20098), 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.
- Moore, R.Y., 1973. Retinohypothalamic projection in mammals: a comparative study. *Brain Res.* 49, 403–409.
- Mora, P.O., Netto, C.F., Graeff, F.G., 1997. Role of 5-HT_{2A} and 5-HT_{2C} receptor subtypes in the two types of fear generated by the elevated T-maze. *Pharmacol. Biochem. Behav.* 58, 1051–1057.
- Naguib, M., Samarkandi, A.H., 2000. The comparative dose–response effects of melatonin and midazolam for premedication of adult patients: a double-blinded, placebo-controlled study. *Anesth. Analg.* 1, 473–479.
- Papp, M., Gruca, P., Boyer, P.A., Mocaër, 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 action of melatonin in mice: involvement of benzodiazepine receptors. *Res. Commun. Chem. Pathol. Pharmacol.* 82, 131–142.
- Redman, R.R., Francis, A.J.P., 1998. Entrainment of rat circadian rhythms by the melatonin agonist S 20098 requires intact suprachiasmatic nuclei but not the pineal. *J. Biol. Rhythms* 13, 39–51.
- Redman, J., Armstrong, S., Ng, K.T., 1983. Free-running activity rhythms in the rat: entrainment by melatonin. *Science* 219, 1089–1091.
- Redman, J.R., Guardiola-Lemaitre, B., Brown, M., Delagrange, P., Armstrong, S.M., 1995. Dose dependent effects of S-20098, a melatonin agonist, on direction of re-entrainment of rat circadian activity rhythms. *Psychopharmacology* 118, 385–390.
- Rocha, B., Rigo, M., Di Scala, G., Sandner, G., Hoyer, D., 1994. Chronic mianserin or eltopazine treatment in rats: effects on the elevated plus-maze test and on limbic 5-HT_{2C} receptor levels. *Eur. J. Pharmacol.* 262, 125–131.
- Tornatzky, W., Miczek, K.A., 1994. Behavioral and automatic responses to intermittent social stress: differential protection by clonidine and metoprolol. *Psychopharmacology* 116, 346–356.

- Tuma, J., Strubbe, J.H., Mocaër, E., Koolhaas, J., 2001. S 20098 affects the free-running rhythms of body temperature and activity and decreases light-induced phase delays of circadian rhythms of the rat. *Chronobiol. Int.* 18, 781–799.
- Van Reeth, O., Olivares, Y., Zhang, F.W., Turek, F.W., Defrance, R., Mocaër, E., 1994. S 20098, a melatonin agonist, phase shifts the circadian clock of mice and Syrian hamster according to a phase-response curve. *Soc. Neurosci. Abstr.* 20, 1440.
- Van Reeth, O., Weibel, L., Olivares, E., Maccari, S., Mocaër, E., Turek, F.W., 2001. Melatonin or a melatonin agonist correct age-related changes in circadian response to an environmental stimulus. *Am. J. Physiol.* 280, 1582–1591.
- Weibel, L., Turek, F.W., Mocaër, E., Van Reeth, O., 2000. A melatonin agonist facilitates circadian clock resynchronization in old hamsters after abrupt shifts in the light–dark cycle. *Brain Res.* 880, 207–211.
- Wiley, J.L., Dance, M.E., Balster, R.L., 1998. Preclinical evaluation of the reinforcing and discriminative stimulus effects of agomelatine (S 20098), a melatonin agonist. *Psychopharmacology* 140, 503–509.
- Ying, S.W., Rusak, B., Delagrange, P., Mocaër, E., Renard, P., Guardiola-Lemaitre, B., 1996. Melatonin analogues as agonists and antagonists in the circadian system and other brain areas. *Eur. J. Pharmacol.* 296, 33–42.
- Yous, S., Andrieux, J., Howell, H.E., Morgan, P.J., Renard, P., Pfeiffer, B., Lesieur, D., Guardiola-Lemaitre, B., 1992. Novel naphthalenic ligands with high affinity for the melatonin receptor. *J. Med. Chem.* 35, 1484.