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Research Article

Effects of the Novel Antidepressant Milnacipran in a Chronic Mild Stress Model of Depression

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ABSTRACT The chronic mild stress (CMS) model of depression may serve as a suitable research tool for studying the action of novel antidepressants (i.e., both efficacy and onset of action). The CMS-induced sub-sensitivity to reward is reversed by chronic treatment with antidepressant drugs. The effect of the serotonin and norepinephrine reuptake inhibitor (SNRI), milnacipran, was investigated on the CMS model in rats in comparison with imipramine. The CMS model of depression consisted in subjecting rats to several mild stressors for a prolonged period of time, which resulted in a decrease in their responsiveness to rewarding stimuli. This deficit was monitored by a decrease in the consumption of a 1% sucrose solution. Stressed and control animals received daily for 5 weeks injections of vehicle, imipramine (10 mg/kg) or milnacipran (3, 10, and 30 mg/kg). CMS caused a decrease in the consumption of the 1% sucrose solution. The deficit in sucrose consumption in stressed animals was reversed by imipramine and milnacipran. The effect of milnacipran was gradual, dose-dependent, and was maintained for one week after stopping drug treatment. Neither imipramine nor milnacipran modified the behavior of control animals. Milnacipran is active in the CMS model of depression as expected from its clinically demonstrated antidepressant effect. Drug Dev. Res. 61:101–106, 2004.

Key words: milnacipran; antidepressant; chronic mild stress; depression model; rats anhedonia

INTRODUCTION

Clinical experience with antidepressant drugs has indicated that they are devoid of mood-elevating effects in normal (i.e., non-depressed) human subjects. On the other hand, animal studies of antidepressants usually involve drug administration to normal animals. Data derived from such studies may, therefore, not be directly relevant to the clinical action of these agents.

For this reason, it is preferable that studies of potential antidepressant drugs use experimental procedures that simulate "depression" in animals [Willner, 1991]. Nonetheless data derived from animal models will be of value only in so far as the models are valid [Willner, 1995]. Important validation criteria are that such models should employ realistic inducing conditions, should model core symptoms of the disorder, and should respond appropriately to antidepressant drugs [McKinney, 1977]. While available pharmacological interaction models generally satisfy the third requirement, very few perform well against all three sets of validating criteria [see reviews of Willner, 1990; Leonard, 1998]. A further difficulty of existing animal models of depression is that they examine phenomena

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of brief duration, and are, therefore, not suitable for investigating the time-course of antidepressant action [Leonard, 1998]. Thus, models should not only be of demonstrated validity, but should also maintain an abnormal state for a prolonged period, during which antidepressant therapy may be administered [Willner et al., 1987]. Of the very few models that meet the criteria outlined above, the Chronic Mild Stress (CMS) model has been most extensively validated and investigated. In this model, rats subjected to a variety of mild stressors for a prolonged period of time show, among other behavioral, biochemical, and physiological impairments, a substantial decrease in their responsiveness to rewarding stimuli [Willner, 1997]. This deficit is usually monitored by a decrease in the consumption of a 1% sucrose solution, but can also be seen in other tests, such as place preference conditioning or intracranial self-stimulation [Willner, 1997]. Since sub-sensitivity to reward appears to reflect anhedonia (inability to experience pleasure), a core symptom of major depressive disorders, the CMS procedure may represent a uniquely useful research tool. Numerous studies have shown that the CMS-induced sub-sensitivity to reward can be reversed by chronic treatment with antidepressant drugs, including tricyclics, atypical antidepressants, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOI), as well as repeated electro convulsive shock (ECS) [Willner, 1987, 1997].

The CMS model has, therefore, proved extremely successful in meeting the requirements of an animal model of depression: it induces a long-lasting state with a high degree of construct, face, and predictive validity [Willner, 1995]. Furthermore, there are no marked discrepancies between the model and the clinic. Finally, it permits studies of the rapidity of onset of antidepressant action.

The serotonin and norepinephrine reuptake inhibitors (SNRI) are a class of antidepressants, without action at post-synaptic receptors, which have recently been introduced [Feeney and Nutt, 1999]. Three SNRI antidepressants are clinically available: venlafaxine, milnacipran, and, more recently, duloxetine. Venlafaxine, and to a lesser extent, duloxetine, exert more marked effects on serotonin reuptake [Bymaster et al., 2001] while milnacipran has a balanced effect on the two neurotransmitters [Moret et al., 1985; Briley and Moret, 1997]. It has been suggested that SNRI antidepressants may have a more rapid onset of action than other antidepressant classes [Montgomery, 1995]. Clinically, however, this remains to be convincingly established. The present study was undertaken to determine the activity of the SNRI, milnacipran, in the CMS model of depression and to investigate its onset of action in comparison with the classical tricyclic antidepressant, imipramine.

MATERIALS AND METHODS

Animals

All animals were housed and handled according to the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985). Male Wistar (Gorzkowska, Warsaw) rats were brought into the laboratory 2 months before the start of the experiment. Except as described below, the animals were housed singly with food and water freely available, and were maintained on a 12 h light/dark cycle (lights on at 08.00) at a temperature of $22 \pm 2^{\circ}C$.

Stress Procedure

The animals were first trained to consume a 1% sucrose solution; training consisted of eight 1-h baseline tests (twice weekly) in which sucrose was presented, in the home cage, following 14 h food and water deprivation; the sucrose intake was measured by weighing pre-weighed bottles containing the sucrose solution at the end of the test (i.e., one-bottle sucrose test). Subsequently, sucrose consumption was monitored, under similar conditions, at weekly intervals throughout the whole experiment.

On the basis of their sucrose intakes in the final baseline test, the animals were divided into two matched groups. One group was subjected to the CMS procedure for a period of 8 consecutive weeks. As shown in Table 1, each week of stress regime consisted of: two periods of food or water deprivation, two periods of 45° cage tilt, one period of intermittent illumination (lights on and off every 2 h), two periods of soiled cage (250 ml water in sawdust bedding), one period of paired housing, two periods of low-intensity stroboscopic illumination (150 flashes/min), and two periods of no stress. All stressors were 10–14 h of duration and were applied individually and continuously, day and night. Control animals were housed in separate rooms and had no contact with the stressed animals. They were deprived of food and water for the 14 h preceding each sucrose measurement, but otherwise food and water were freely available in the home cage.

Drug Administration

On the basis of their sucrose intakes following 2 weeks of stress, both stressed and control animals were divided into matched subgroups ($n = 8$), and for subsequent 5 weeks they received daily intraperitoneal injections of vehicle (1 ml/kg, distilled water),

TABLE 1. Schedule of a Weekly Stress Regimen[a]

Monday morning	Stroboscopic illumination (150 flashes/min)
Monday evening	Soiled cage (250 ml water in sawdust bedding)
Tuesday morning	Cage cleaning, followed by no stress
Tuesday 08.00 pm	Food and water deprivation
Wednesday 10.00 am	Sucrose test, followed by food or water deprivation
Wednesday evening	Cage tilting (45 degrees backwards)
Thursday morning	Intermittent illumination (lights on and off every 2h)
Thursday evening	Soiled cage
Friday morning	Cage cleaning, followed by water deprivation
Friday evening	Grouped housing (two animals in one cage)
Saturday morning	Cage tilting
Saturday evening	Stroboscopic illumination
Sunday morning	Food deprivation
Sunday evening	Food return, followed by no stress

[a]See text for further details.

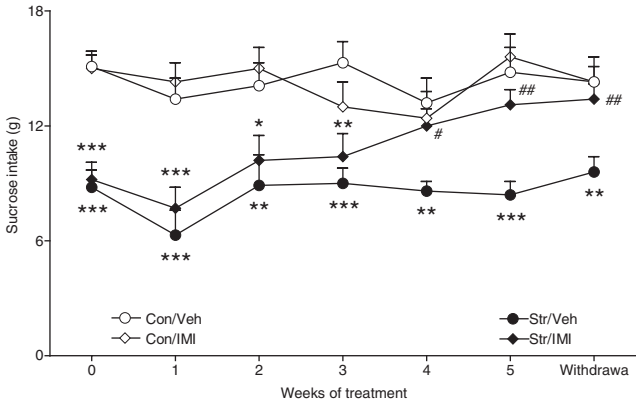

Fig. 1. Effect of chronic treatment with vehicle (Veh, 1 ml/kg/day) and imipramine (IMI, 10 mg/kg/day) on the consumption of 1% sucrose solution in controls (open symbols) and in animals exposed to CMS (closed symbols). Treatment commenced following 3 weeks of stress. Values are means ± S.E.M. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; relative to vehicle- or drug-treated control groups. $^{\#}P<0.05$; $^{\#\#}P<0.01$; relative to drug-treated stressed animals at Week 0.

imipramine (10 mg/kg), or milnacipran (3, 10, and 30 mg/kg). The dose of imipramine chosen for this study has been shown to cause stable and comparable effects after chronic treatment in previous CMS studies using the same experimental protocol. The drugs were administered at approximately 10:00 and the sucrose measurements were carried out 24 h after the last drug injection. After 5 weeks, all treatments were terminated and one additional sucrose test was carried out after one week of withdrawal. Stress was continued throughout the period of treatment and withdrawal.

Statistics

Changes in sucrose consumption during the 5 weeks of vehicle and drug administrations in control and stressed animals were analyzed by multiple analysis of variance with two between-subject factors (group and treatments) and successive sucrose measurements as within-subject factor. The Fisher's LSD test was used for post-hoc comparisons of means. $P<0.05$ was considered statistically significant.

RESULTS

Effects of CMS

CMS caused a gradual decrease in consumption of 1% sucrose solution. In the final baseline measurement, all animals drank approximately 16 g of sucrose solution. Following an initial 2 weeks of stress, intakes remained at a similar level in controls (mean intakes at week 1 and 2 of initial stress: 14.4 and 15.6, respectively) but fell to approximately 9 g in stressed animals (mean intakes at week 1 and 2 of initial stress: 10.4 and 9.1, respectively). Consequently, before the treatments were started (i.e., at Week 0) the sucrose consumption in control and stressed animals was significantly different [Group effect: $F(1,70)=120.224$; $P<0.001$]. This difference between control and stressed animals receiving vehicle persisted at a similar level for the remainder of the treatment period, which is reflected in significant Group effect [$F(1,14)=32.569$; $P<0.001$], and non-significant Weeks effect and Group × Weeks interaction [$F(5,70)=1.795$ and 0.546, respectively] (Fig. 1).

Effect of Imipramine and Milnacipran

Imipramine had no significant effect on sucrose intake in control animals [Treatment effect: $F(1,14)=0.010$, Weeks effect: $F(5,70)=1.723$, interaction: $F(5,70)=0.871$; all non-significant] and caused a complete restoration of sucrose drinking in stressed animals [Treatment effect: $F(1,14)=4.971$; $P<0.05$, Weeks effect: $F(5,70)=8.655$; $P<0.001$, interaction: $F(5,70)=3.912$; $P<0.01$]. In consequence, overall effect of 5 weeks of imipramine administration resulted in significant effects of Group [$F(1,28)=34.690$; $P<0.001$] and Treatment [$F(1,28)=3.989$; $P<0.05$] and Group × Treatment interaction [$F(1,28)=5.079$; $P<0.05$]. Post hoc analysis revealed

that imipramine-induced enhancement of sucrose intakes, relative to Week 0 scores, reached statistical significance after four ($P = 0.048$) and five ($P = 0.006$) weeks of treatment (Fig. 1), giving rise to significant weeks effect [$F(5,70) = 8.655$; $P < 0.001$] and Weeks × Treatment interaction [$F(5,70) = 3.912$; $P < 0.01$). As a result, at the end of treatment period (Week 5) the amount of sucrose solution drank by these animals was comparable to that of vehicle-treated controls ($P = 0.312$) and significantly higher than that of vehicle-treated stressed animals ($P = 0.004$). Finally, sucrose intakes in control and stressed animals administered imipramine were maintained at a similar level one week after withdrawal from the drug (Week 5 vs. Withdrawal: Group, Weeks effects, and interaction: $F(1,14) = 1.138$, 1.146, and 0.418, respectively; all non-significant).

Milnacipran did not significantly affect the consumption of sucrose solution in non-stressed control animals but gradually increased sucrose consumption in stressed animals (Fig. 2), resulting in significant effects of Group [$F(1,56) = 58.889$; $P < 0.001$] and Treatment [$F(3,56) = 3.995$; $P < 0.05$] and Group × Treatment × Weeks interaction [$F(12,224) = 2.080$; $P < 0.01$]. The three doses of milnacipran differed in the onset of their action and efficacy. Thus, the effect of 3 mg/kg, although apparent at the end of treatment period, did not give rise to significant effects of Treatment [$F(1,14) = 3.313$; $P = 0.09$], Weeks [$F(4,56) = 1.885$; $P = 0.126$], and interaction [$F(4,56) = 2.133$; $P = 0.09$]. The dose of 10 mg/kg caused significant Treatment and Weeks effects ($F(1,14) = 4.666$; $P < 0.05$ and $F(4,56) = 5.032$; $P < 0.01$, respectively] but not interaction [$F(4,56) = 2.143$; $P = 0.08$]. The highest dose of 30 mg/kg was most effective and caused all significant effects and interaction [Treatment: $F(1,14) = 6.966$; $P < 0.05$, Weeks: $F(4,56) = 6.446$; $P < 0.001$ and interaction: $F(4,56) = 2.966$; $P < 0.05$]. Consequently, post hoc analysis revealed that, when compared to the intakes in drug-treated stressed groups at Week 0, the increase of sucrose consumption by milnacipran administered at the lowest dose of 3 mg/kg did not reach statistical significance at any time point during treatment although the slow rise in consumption seen throughout the treatment period became significant one week after the treatment was withdrawn. The dose of 10 mg/kg increased sucrose intakes significantly at Week 5 ($P = 0.028$), and the dose of 30 mg/kg caused significant effects after 3 ($P = 0.030$), 4 ($P = 0.049$), and 5 ($P = 0.013$) weeks of treatment. Thus, after 5 weeks of treatment with 10 and 30 mg/kg (but not 3 mg/kg) of milnacipran, the stressed animals drank significantly more sucrose solution than the stressed group treated with vehicle (3.0 mg: $P = 0.070$; 10 mg: $P = 0.015$; 30 mg: $P = 0.007$) (Fig. 2).

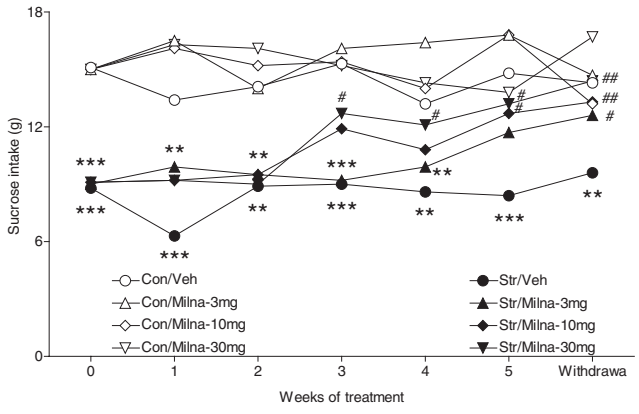

Fig. 2. Effect of chronic treatment with vehicle (Veh, 1 ml/kg/day) and milnacipran (Milna, 3, 10, and 30 mg/kg/day) on the consumption of 1% sucrose solution in controls (open symbols) and in animals exposed to CMS (closed symbols). Treatment commenced following 3 weeks of stress. Values are means; standard errors have been omitted for clarity. $^{**}P < 0.01$; $^{***}P < 0.001$; relative to vehicle- or drug-treated control groups. $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; relative to drug-treated stressed animals at Week 0.

It is noteworthy that 3-way analysis of data obtained from animals receiving imipramine (10 mg/kg) and milnacipran at the most active dose of 30 mg/kg revealed non-significant Treatment effect [$F(1,28) = 0.746$; non-significant] and Treatment × Weeks [$F(4,112) = 1.996$; non-significant] and Treatment × Group × Weeks [$F(4,112) = 1.080$; non-significant] interactions. Also at the end of the treatment period (Week 5), the consumption of sucrose solution by these animals did not differ significantly (imipramine vs. milnacipran: $P = 0.969$). These data suggest that both the time-course and the magnitude of the effect of milnacipran in the CMS model are comparable to those of imipramine.

As seen with imipramine, one week of withdrawal from treatment with milnacipran did not significantly affect the sucrose consumption in both control and stressed groups (Week 5 vs. Withdrawal: Group, Weeks effects and interaction: $F(1,46) = 2.18$, 0.188, and 1.062, respectively; all non-significant).

At the start of the study, all animals weighed approximately 360 g, and after initial 2 weeks of stress (Week 0), the body weight of controls (393 ± 6 g) and stressed (382 ± 7 g) did not significantly differ [$F(1,78) = 1.233$; non-significant]. As shown in Table 2, at the end of the treatment period the vehicle-treated control animals were slightly but not significantly lighter than the vehicle-treated stressed animals. Imipramine significantly decreased the body weights of the stressed

TABLE 2. Body Weights (g) After 5 Weeks of Treatment[a]

Groups	Controls	Stressed
Vehicle	436±20	451±13
Imipramine (10 mg/kg)	433±11	381±12*
Milnacipran (mg/kg)		
3	460±19	418±19
10	431±15	420±21
30	408±19	410±14

[a]Values are means±S.E.M.
*$P<0.01$; relative to vehicle-treated stressed group.

group (Table 2). Milnacipran had no significant effect on the body weights in control and stressed animals at the three doses used (Table 2).

DISCUSSION

The CMS model was developed in an attempt to model stressful conditions that may act as trigger factors for depression in humans. Consistent with previous reports [for review, see Willner, 1997], the present study shows that the CMS procedure decreases the sensitivity to reward as indicated by a reduction in the consumption of a 1% sucrose solution. Normal sucrose consumption is restored when the rodent is treated chronically with an antidepressant [Willner, 1997].

The tricyclic antidepressant, imipramine, has been repeatedly shown to reverse the stress-induced decrease in the consumption of 1% sucrose [Monleon et al., 1995; Papp and Wieronska, 2000]. Sucrose preference also returns to normal after 2–4 weeks of treatment with the tricyclic antidepressant, desipramine [Willner et al., 1987]. The reversible inhibitor of monoamine oxidase type A (RIMA), brofaromine, effectively restores the normal levels of sucrose drinking in the sensitivity to reward [Papp et al., 1996].

Several SSRIs have been tested in the CMS model of depression. Sertraline [Marona-Lewicka and Nichols, 1997] is effective in restoring normal sucrose consumption. Citalopram and escitalopram, the active S-enantiomer of citalopram, are also active in reversing the stress-induced deficit in drinking a sweet solution [Montgomery et al., 2001]. Moreover, the onset of action of escitalopram in the CMS model has been found faster than that of citalopram, and this shorter delay in the onset of antidepressant activity of escitalopram has been confirmed in clinical studies [Montgomery et al., 2001].

Recently, the SNRI antidepressant, venlafaxine, has been shown in the CMS model of depression to enhance the sucrose consumption [Millan et al. 2001]. Taken together, the above-reviewed studies suggest that the CMS procedure is sensitive to a wide variety of clinically active antidepressants with different mechanisms.

The present study shows that the SNRI, milnacipran, is active in the CMS model of depression. Milnacipran progressively and dose-dependently reverses the deficit in sucrose consumption in stressed animals and, like all the drugs mentioned above, it does not affect the behavior of control animals. The effect of milnacipran is maintained for at least one week after cessation of the treatment and no signs of withdrawal are observed in either stressed or control animals. These results are consistent with clinical observations that milnacipran is well tolerated and does not cause any withdrawal effects or changes in body weight [Puech et al., 1997].

At 3 mg/kg, the effect of milnacipran is not significant during the treatment period although it becomes significant when measured one week after withdrawal. This suggests that the slow onset of antidepressant action seen in this model probably reflects a mechanism that, once triggered by the antidepressants, continues to progress with its own kinetics independently of the presence of the drug. A similar mechanism has been suggested based on clinical data [Stahl et al., 2001].

The magnitude and time-course of action of the most active dose of milnacipran (30 mg/kg) in the chronic stress model were comparable with those of imipramine. Clinically, milnacipran is used at 100 mg/day and imipramine at 120–150 mg/day. The doses used in this study were initially chosen to represent the same ratio between the compounds. In practice, however, it was not possible to give imipramine at a dose equivalent to milnacipran (which would have been 40–50 mg) for reasons of toxicity. The results of the present study, therefore, confirm clinical studies suggesting a marked similarity between the two treatments.

In summary, the clinically effective antidepressant, milnacipran, administered chronically, restores to normal values the deficit in the consumption of sucrose solution induced by the CMS model of depression. The magnitude of the effect and the rate of onset of action are essentially similar to the tricyclic antidepressant imipramine. These results provide further support for the value of the CMS paradigm in modeling an important aspect of human depressive disorders.

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