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Exploratory behaviour and grooming after repeated restraint and chronic mild stress: effect of desipramine

Paolo S. D'Aquila*, Alessandra T. Peana, Vittorio Carboni, Gino Serra

Dipartimento di Scienze del Farmaco, Università degli Studi di Sassari, Via Muroni 23 / a, I-07100 Sassari, Italy

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Abstract

In a previous study, we have recently shown that chronic treatment with desipramine either reduced or potentiated the locomotor response to the dopamine D_2-like receptor agonist quinpirole, a behavioural response mediated by the mesolimbic dopamine system, depending on whether the animals were subjected, respectively, to repeated restraint or to chronic mild stress (different stressors randomly presented). In this study, we examined the interaction between prolonged exposure to either repeated restraint stress or chronic mild stress with the chronic administration of the antidepressant desipramine on two spontaneous behaviours, in which an involvement of the mesolimbic dopamine system has been suggested: novelty-induced exploratory activity and grooming. Exploratory activity in the open field was reduced by chronic mild stress regardless of the drug treatment, while it was not influenced by restraint stress. Desipramine reduced exploratory activity in rats subjected to restraint stress. Restraint stress increased grooming and desipramine reversed this effect, while increasing grooming in the chronic mild stress group. These findings suggest that antidepressants exert their effect by opposing the modifications induced by stress. The available experimental evidence is consistent with the hypothesis that an important role in the observed behavioural changes is played by the mesolimbic dopamine system.

Keywords: Antidepressant drug; Desipramine; Grooming; Locomotor activity; Mesolimbic dopamine system; Open field; Restraint stress; Chronic mild stress

1. Introduction

In a recent study (D'Aquila et al., 1997), we observed that exposure of rats to two different stress regimes influenced in an opposite manner the effect of chronic treatment with the tricyclic antidepressant desipramine on the locomotor response to the dopamine D_2-like receptor agonist quinpirole (Sokoloff et al., 1992), a behavioural response mediated by the stimulation of dopamine receptors in the mesolimbic dopamine system (Kelly et al., 1975). In particular, chronic treatment with desipramine in rats subjected to repeated restraint stress reduced the locomotor response to quinpirole, while in rats exposed to chronic mild stress (see Willner et al., 1992) increased it. In previous studies, repeated restraint and chronic mild stress have been reported, respectively, to increase and to decrease the locomotor response to dopamine agonists (see Cabib and Puglisi-Allegra, 1996; Papp et al., 1993; Willner et al., 1992). These observations prompted us to suggest that antidepressants influence the mesolimbic dopamine system sensitivity in a direction which is opposite to that induced by stress, at least as far as the neural circuits mediating locomotor activity are concerned.

On the basis of these observations, we set ourselves the aim to determine whether the bi-directional anti-stress effect of antidepressants detectable by challenging the subjects with a dopamine agonist was paralleled by behavioural changes under more natural conditions.

To explore this possibility, we decided to study the interaction between desipramine and either repeated restraint or chronic mild stress on a spontaneous behaviour, in which an involvement of the mesolimbic dopamine system has been suggested, i.e. novelty-induced exploratory behaviour in the open field (Fink and Smith, 1980). Moreover, significant differences between the groups emerged also in the grooming behaviour displayed

* Corresponding author. Tel.: +39-079-228739; fax: +39-079-228712.
E-mail address: dsfpaolo@ssmain.uniss.it (P.S. D'Aquila).

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by the animals in the open field arena, revealing a picture surprisingly similar to that observed in the quinpirole locomotor response experiment. Grooming behaviour can be observed after exposure to a novel enviroment (see Ferrari et al., 1992). As far as dopaminergic mechanisms are concerned, an involvement of the mesolimbic dopamine system in novelty-induced grooming has been suggested (Prinssen et al. 1994). Selective dopamine D_1/D_5 receptor agonists elicit an intense grooming (Watchel et al., 1992), while dopamine D_2 receptor agonists reduce this behaviour (Ferrari et al. 1992).

2. Materials and methods

The present study has been carried out in accordance to the 'Principles of laboratory animal care' (NIH publication No. 85-23, revised 1985).

2.1. Subjects and drugs

Experiments were performed on male Wistar rats (Charles River, Como, Italy), initially weighing 250–300 g. The animals were housed 2–3 per cage in air-conditioned rooms (temperature: 21–23°C; humidity: 50–55%) with a 24 h light/dark cycle (light from 0800 to 2000 h) and with water and a standard laboratory diet ad libitum. The animals were allowed 3 weeks of acclimatization after arrival, before commencing stress exposure and chronic treatment.

Desipramine HCl (Sigma, St. Louis, USA) was dissolved in distilled water.

2.2. Stress and chronic treatment

The animals ($N = 64$) were allocated into three groups ($n = 21–22$): (1) no-stress (controls), (2) restraint stress and (3) chronic mild stress. Each group was further divided into two groups: desipramine, receiving a daily injection of desipramine HCl 10 mg/kg i.p. in a volume of 1 ml/kg, and vehicle, receiving a daily injection of the same volume of distilled water. Injections were performed between 0830 and 1000 h.

Animals in the restraint stress group were immobilized into a perspex semicylinder (base: cm 10×20; radius: cm 4) for 1 h daily, immediately after the drug treatments (see below).

Chronic mild stress consisted in the exposure to the following stressors in a random order: food deprivation (twice a week), water deprivation (twice a week), crowding (once a week), change of cage mates (twice a week), isolation (once a week), light switched on during the dark time (once a week), soiling of the cage bedding (once a week); exposure to a single stressor lasted from 8 to 18 h.

Stress administration and chronic drug treatment were commenced simultaneously and carried out for 7 weeks.

2.3. Open field

The open field consisted in a white square (cm $100 \times 100 \times 20$ h) divided into 25 identical sectors (cm 20×20) by black stripes. The animals were placed in the central sector and their activity recorded for 5 min by a video camera. A motility count was considered when an animal crossed a sector border with both its hindlimbs. The total duration of grooming episodes was recorded. Experimental observations were performed 24 h after the end of treatments.

Experiments were performed between 0900 and 1700 h in a soundproof room. The videotapes were analysed by observers unaware of the treatment received by the subjects.

2.4. Data analysis

Results were analysed by analysis of variance (ANOVA), supplemented by *F*-tests for contrasts, using the appropriate analysis of variance error term (Winer, 1971). Analysis of behavioural data involved two between groups factors, stress (with 2 levels: no-stress and stress) and desipramine (with two levels: vehicle and drug). From the analysis, the values laying outside a range of two standard deviations from the mean of their group were excluded. Analysis of weight data involved two between groups factors, stress (with three levels: no-stress, restraint stress and chronic mild stress) and desipramine (with two levels: vehicle and drug) and a within group factor, time (with two levels: pre- and post-treatment).

3. Results

3.1. Open field: exploratory activity

3.1.1. Restraint stress

ANOVA showed a significant main effect of the factor drug [$F(35,1) = 4.41$, $P = 0.042$] and a significant interaction between the factors stress and drug [$F(35,1) = 4.77$, $P = 0.035$]. Further analysis (*F*-tests for contrasts) revealed that desipramine reduced the motility counts in the restraint stress group [$F(35,1) = 8.98$, $P = 0.0049$] (Fig. 1).

3.1.2. Chronic mild stress

ANOVA showed a significant main effect of the factor stress [$F(35,1) = 6.76$, $P = 0.013$], due to a decrease in motility counts regardless of the drug treatment. Indeed, both the main effect of the factor drug and the interaction between drug and stress were not significant (Fig. 1).

3.2. Open field: grooming time

3.2.1. Restraint stress

ANOVA revealed a significant stress x drug interaction [$F(27,1) = 6.34$, $P = 0.018$], while no significant main

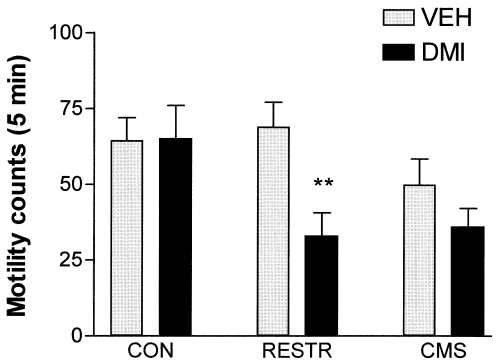

Fig. 1. Exploratory activity in the open field. CON = no stress; RESTR = restraint stress; CMS = chronic mild stress; VEH = vehicle; DMI = desipramine. Each value represents mean ± SEM from 8–11 subjects (CON-VEH, $n = 11$; CON-DMI, $n = 9$; RESTR-VEH, $n = 10$; RESTR-DMI, $n = 9$; CMS-VEH, $n = 11$; CMS-DMI, $n = 8$). The animals were placed in the open field arena for 5 min. $^{**}P = 0.0049$: effect of desipramine with respect to the corresponding vehicle-treated group (ANOVA followed by *F*-test for contrasts).

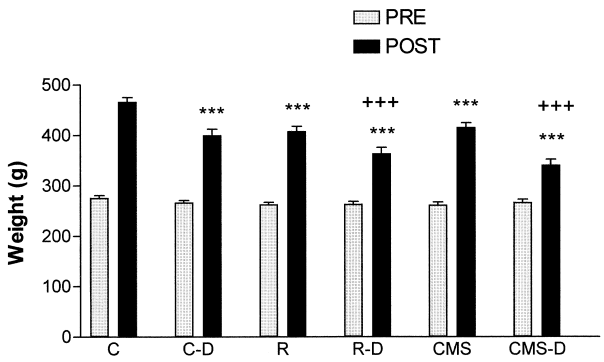

Fig. 3. Weight gain along the course of stress and desipramine administration. PRE: beginning of the treatment; POST: end of treatment. C: controls; D: desipramine; R: restraint stress; CMS: chronic mild stress. Each value represents mean ± SEM from 8–11 subjects (CON-VEH, $n = 11$; CON-DMI, $n = 9$; RESTR-VEH, $n = 10$; RESTR-DMI, $n = 9$; CMS-VEH, $n = 11$; CMS-DMI, $n = 10$). All groups showed a significant increase of weight ($P < 0.001$). $^{***}P < 0.001$ with respect to the non-stressed control group. $^{+++}P < 0.001$ effect of desipramine in stressed animals with respect to the corresponding vehicle treated group (ANOVA followed by *F*-test for contrasts).

effect of any of the two factors was present. Further analysis (*F*-tests for contrasts) revealed the following effects: restraint stress increased the time spent in grooming [$F(27,1) = 7.04$, $P = 0.013$] in the vehicle-treated group and desipramine reduced the number of grooming episodes in the restraint stress group [$F(27,1) = 4.31$, $P = 0.047$] (Fig. 2).

3.2.2. Chronic mild stress

ANOVA revealed a significant main effect of the factor stress [$F(30,1) = 8.18$, $P = 0.007$] and drug [$F(30,1) = 18.17$, $P = 0.00018$], with an interaction between the two on the verge of statistical significance [$F(30,1) = 3.76$, $P = 0.061$]. Further analysis (*F*-tests for contrasts) revealed a significant increase in the grooming time induced by desipramine in the chronic mild stress treated animals [$F(30,1) = 20.48$, $P = 0.000089$] (Fig. 2).

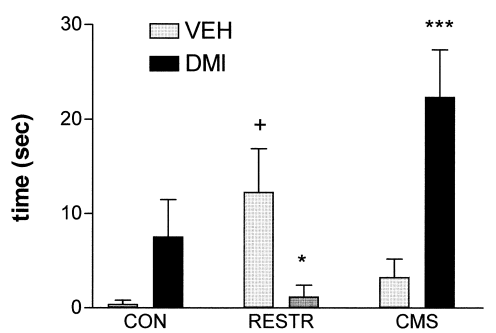

Fig. 2. Grooming behaviour in the open field: time. CON = no stress; RESTR = restraint stress; CMS = chronic mild stress; VEH = vehicle; DMI = desipramine. Each value represents mean ± SEM from 6–11 subjects (CON-VEH, $n = 9$; CON-DMI, $n = 7$; RESTR-VEH, $n = 10$; RESTR-DMI, $n = 6$; CMS-VEH, $n = 10$; CMS-DMI, $n = 8$). The animals were placed in the open field arena for 5 min. $^{*}P = 0.047$, $^{***}P = 0.000089$: effect of desipramine with respect to the corresponding vehicle-treated group; $^{+}P = 0.013$: effect of stress with respect to the corresponding no-stress group (ANOVA followed by *F*-test for contrasts).

3.3. Body weight gain

ANOVA revealed a main effect of the factors stress [$F(2,53) = 11.3$, $P < 0.001$], drug [$F(1,53) = 30.6$, $P < 0.001$], and time [$F(1,53) = 1516$, $P < 10E-6$], while no significant interaction between either stress and drug [$F(2,53) = 0.775$, $P = 0.46$, n.s.] or stress, drug and time [$F(2,53) = 2.58$, $P = 0.085$, n.s.] was present. All the groups showed a significant increase in weight between the beginning and the end of treatments. At the end of the treatments, all the groups receiving desipramine, stress (either restraint or chronic mild stress), and the two treatments combined showed a weight significantly lower with respect to the no stress group treated with desipramine. Treatment with desipramine resulted in a further decrease of weight, both in the restraint stress and in the chronic mild stress group. No difference in weight gain was observed between the animals receiving the two different stress schedules (Fig. 3).

4. Discussion

Chronic mild stress, but not restraint stress, influenced exploratory activity, resulting in a reduction of the motility counts. This observation is consistent with an earlier study performed by Katz et al. (1981), involving exposure to a stress regime of which the chronic mild stress model is a development (Willner et al., 1992). Chronic treatment with desipramine influenced exploration only in animals subjected to restraint stress, resulting in a reduction of motility

counts. The latter observation parallels the reduction in the locomotor response to quinpirole induced by chronic treatment with desipramine in animals subjected to repeated restraint stress (D'Aquila et al., 1997), and might be interpreted as a reduced responsiveness of the mesolimbic dopamine system to exposure to novelty.

Relevant differences in grooming behaviour were observed across the groups. In particular, grooming was increased by exposure to the restraint stress regime, and this effect was reversed by desipramine. On the contrary, desipramine increased grooming in the chronic mild stress group. These results resemble very closely the picture observed in the locomotor response to quinpirole (D'Aquila et al., 1997), showing the ability of desipramine either to increase or to decrease this behavioural response, depending on the stress schedule to which the animals were subjected. It would be tempting to suggest that the neurobiological substrate underlying the interaction between stress and desipramine in these behavioural responses might be the same, i.e. the mesolimbic dopamine system. However, it is well known that dopamine D_2-like receptor agonists, in contrast to dopamine D_1 receptor agonists (Watchel et al., 1992), reduce grooming (Ferrari et al., 1992). Therefore, an increase in the sensitivity to dopamine D_2-like receptor stimulation, if anything, should be paralleled by changes in grooming behaviour in the opposite direction with respect to the one we observed.

A possible speculative solution to this problem might be offered by some observations. Experimental evidence has been produced showing on the one hand that chronic treatment with antidepressants increase both the locomotor response to a dopamine D_3 receptor agonist and the density of these receptors in areas of the mesolimbic dopamine system (Maj et al., 1998), and, on the other hand, that the increased sensitivity to dopamine D_2-like receptor agonists might coexist with an increased level of neurotransmission at the dopamine D_1 receptor level (Serra et al., 1990). More recently, it has been shown that dopamine D_3 receptor stimulation is able to potentiate the grooming behaviour induced by the administration of a dopamine D_1 receptor agonist (Noel et al., 1998). Taking together these findings, a hypothesis might be suggested: that the changes in neurotransmission at the dopamine D_1 receptor level and the changes in dopamine D_3 receptors induced by the chronic antidepressant treatment might induce changes in grooming behaviour, which parallel the changes in the locomotor response to the dopamine D_2-like receptor agonist quinpirole.

This hypothesis is also consistent with the observation that an involvement of the mesolimbic dopamine system, which is the biological substrate of the locomotor response to dopamine receptor agonists (Kelly et al., 1975), has been shown to be involved in grooming behaviour (Prinssen et al., 1994).

Reduction of body weight gain can be considered as an index of the severity of a stress regime (e.g. Armario et al., 1983; Cure, 1989; Konarska et al., 1990). Since the two different stress schedules reduced body weight gain in a similar way, their different behavioural effects do not seem to be due to a difference in stress severity.

Regardless of the interpretation of the data in terms of neurobiological substrates, which at this stage is highly speculative and far from conclusive, our data show that the bi-directional anti-stress effect of desipramine that we previously observed after quinpirole challenge is paralleled by changes in behaviour under more natural conditions. Taken together, these findings suggest that antidepressants exert their effect by opposing the modifications induced by stress. The available experimental evidence is consistent with the hypothesis that an important role in the observed behavioural changes is played by the mesolimbic dopamine system.

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