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Research report

Augmentation of the behavioural effects of desipramine by repeated immobilization stress

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ABSTRACT

The present report provides evidence that repeated immobilization stress (RIS) induced a noradrenergic-dependent depressive-like behaviour and an augmented behavioural response to desipramine (DMI), a noradrenaline reuptake inhibitor (NRI), in the forced swimming test (FST). The present results show that RIS decreased the baseline of climbing behaviour in the FST. Whereas subchronic administration of DMI (10 mg/kg, three times in a 24 h period) induced a significantly higher increase in climbing behaviour on repeatedly stressed rats compared to controls. The results also show that the concomitant administration of the low dose of DMI (3 mg/Kg) during the RIS fully prevented the decrease of climbing behaviour induced by RIS, without exerting behavioural effects in control rats, further supporting an augmented response to the DMI antidepressant effects in the repeatedly stressed rats. In conclusion, our data indicate that RIS not only changes the behavioural responses in the FST but also increases the antidepressant effects of DMI.

1. Introduction

Depression is a complex disorder, and the mechanisms underlying its pathogenesis remain unrevealed. Clinical and preclinical evidence indicates that stressful life events and chronic stress are risk factors for developing depression [1,15,16]. Various chronic stress models such as chronic mild stress and social defeat have been developed to replicate depressive symptoms or identify neurobiological substrates underling human depression [34,31]. Chattarji and collaborators, using repeated immobilization stress (RIS), have shown that RIS induces long-lasting increase of dendritic arborization in the basolateral amygdala neurons and anxiety-like behaviour; meanwhile, induces atrophy and debranching in CA3 neurons of the hippocampus [32,33]. RIS also induces opposite effects on noradrenaline (NA) turnover in the central nucleus of the amygdala (CeA) and paraventricular nucleus of the hypothalamus (PVN; [19,20]). In addition, our group has shown that RIS induces a long-lasting increase of corticotrophin releasing hormone (CRH) immunoreactivity in the extended amygdala. This increase of CRH immunoreactivity is prevented by DMI, a selective NA reuptake inhibitor [27]. Overall, RIS recapitulates some features of human depression such as smaller volumes of the hippocampus [28], amygdala hyperactivity [11], anxiety and CRH system hyperactivity [8].

The FST originally described by Porsolt et al. [23,24] is the most reliable method to assess antidepressant activity in rodents. In the FST the animal undergoes two trials (24 h apart) in which it is placed into a cylinder of water, and active escape behaviour and immobility time are measured. The most common observation is that animals become immobile faster on the second exposure. Antidepressant treatments decrease immobility time, delay its onset and/or increase active escape behaviours [2,4]. Lucki and collaborators [10] developed a behaviour sampling technique to score the active behaviours climbing and swimming, and the time of immobility. This technique showed that selective NRI's such as DMI increased climbing, without affecting swimming, and selective serotonin reuptake inhibitors (SSRI's) such as fluoxetine and sertraline increased swimming without affecting climbing [5,7,9,10,21].

In the present work, we studied the effect of RIS and DMI administration on the behavioural responses in the FST, using the behaviour sampling technique [10]. In order to achieve this goal, we exposed repeatedly stressed male rats to the FST, focusing our analysis on the effects of RIS on the active escape behaviours, climbing and swimming, to assess putative roles for the noradrenergic and/or serotoninergic system on RIS- and DMI-dependent effects.

2. Materials and methods

2.1. Drugs and chemicals

DMI (Sigma, St. Louis, MO, USA) was dissolved in sterile deionized water, prepared freshly each day and administered intraperitoneally (i.p.) at 3 or 10 mg/kg.

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DMI doses were selected based on previous studies showing antidepressant-like effect in the FST [25].

2.2. Subjects

Male Sprague–Dawley rats from the Pontificia Universidad Católica de Chile animal care facility; weighing 210–230 g at the start of the experiment were used. Rats were maintained on a 14-h light, 10-h dark schedule (lights on between 07:00 and 21:00), food and water was available *ad libitum*. The body weight of each rat was daily monitored. All experiments were conducted in accordance with institutional (P. Universidad Católica de Chile) and international guidelines (Guide for the Care and Use of Laboratory Animals, NIH Publications No. 80-23, revised 1996).

2.3. Stress protocol

RIS consisted of a daily session of immobilization (15 days, 2 h/day, 10:00–12:00 h) in rodent plastic bags without access to either food or water as described by Santibañez et al. [27]. At the end of each experimental protocol, rats were deeply anesthetized with chloral hydrate (400 mg/kg) and both adrenal glands were weighed as previously described [26].

2.4. Forced swim test protocol

The FST was performed using the protocol previously described by Detke et al. [10]. On day 16 (1 day after the last stress session), rats were introduced into transparent acrylic cylinders (45 cm height, 20 cm in diameter) containing water (23–25 °C) up to 30 cm. An initial 15-min pretest followed by a 5-min test 24 h later was performed between 14:00 and 19:00 h. After each swimming session, rats were removed from the cylinders, dried and returned to their home cages. The behaviour during pretest and test sessions was recorded on videotape (Sony color video camera). The water in the cylinders was changed after every other trial.

2.5. Forced swim test behavioural analysis

Each recorded 5-min test session of FST was analyzed to score behaviours [10]. Behavioural scoring was done by three ratters blind to the treatment conditions. Each 5-s period during the test session ratters scored immobility, swimming and climbing. Immobility corresponds to float in the water making only those movements necessary to keep the head above the water; swimming corresponds to active swimming motions between quadrants of the cylinder, more than necessary to merely keep the head above water and climbing corresponds to active movements with forepaws in and out of the water, usually directed against the walls [10].

2.6. Locomotor activity

Rats were placed individually in the locomotor testing cage (47 cm × 15 cm × 26 cm) equipped with two pairs of infrared sensors. The locomotor activity was determined essentially as previously described [12].

2.7. Experimental protocols

Experimental protocol 1: Rats were randomly assigned to one of two experimental groups: RIS and *naïve* controls. *Naïve* control animals were daily manipulated for the same 15 days to avoid stress induced by handling. On day 16, both experimental groups were subjected to the FST.

Experimental protocol 2: Rats subjected to RIS protocol and its respective *naïve* controls were treated with DMI (3 or 10 mg/kg, once a day at 09:00 AM), or vehicle (deionized water, once a day at 09:00 AM) during 15 days. On day 16, all groups were subjected to the FST.

Experimental protocol 3: Rats subjected to RIS protocol and its respective *naïve* controls were subjected to the swim pretest of the FST. Then, DMI (10 mg/kg) or and vehicle (deionized water) were administered 23.5, 5 and 1 h before the 5-min swim test [10].

2.8. Statistical analysis

For behavioural analysis 6–22 animals in each experimental group were used. The data corresponds to the mean ± SEM of each scored behaviour (immobility, climbing, swimming and locomotor activity), and was analyzed using unpaired *t*-test to compare *naïve vs.* RIS. The effect of DMI treatments on FST behaviours, locomotor activity and adrenal weight were analyzed using a two-way analysis of variance (ANOVA) followed by a Bonferroni *post hoc* test, with DMI and RIS as factors. The effect of vehicle was also analyzed using a two-way ANOVA with vehicle and RIS as factors. The temporal course of body weight gain was analyzed by a two-way ANOVA with treatments and time as factors. The statistical analysis was carried out with GraphPad Prism (GraphPad Software, San Diego, CA, USA).

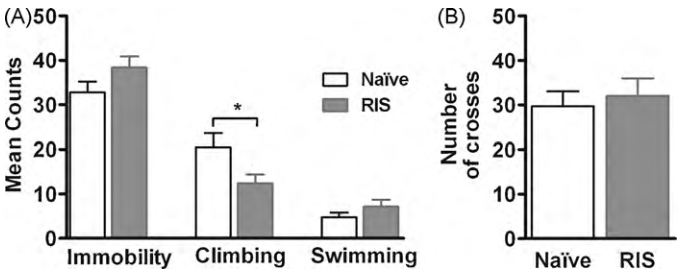

Fig. 1. Effect of RIS on behavioural activity in the FST and locomotor activity. (A) Two days after the end of experimental protocol 1, all rats were subjected to the 5-min test of forced swimming. The data corresponds to the mean ± SEM counts of immobility, climbing and swimming obtained from 15 independent rats for each group. (B) One day after the end of experimental protocol 1 and before the 15 min pretest of forced swimming, the rats were subjected to the locomotor activity test. The data corresponds to the mean ± SEM of the number of crosses obtained from 15 independent rats for each group. $^{*}p < 0.05$ RIS compared to naïve.

3. Results

3.1. Effect of RIS on active behaviours in the FST and in locomotor activity

RIS significantly decreased climbing behaviour ($p < 0.05$), without changing immobility and swimming behaviour (Fig. 1A). No significant changes between groups were observed in locomotor activity (Fig. 1B, $p = 0.653$). A significant increase of immobility score on repeated stressed rats was observed when the scores obtained from RIS and RIS vehicle rats were pooled (see supplemental Fig. 2). RIS also increases adrenal weight and decreases the body weight gain (see supplemental Table 1 and Fig. 1).

3.2. Effect of DMI administration during the stress period in the FST

Fig. 2 shows the effect of DMI administration to naïve and RIS rats. DMI significantly decreased immobility score on repeated stressed rats at both doses studied (3 and 10 mg/kg), but only at the higher dose decreased immobility in control rats. The two-way ANOVA showed a significant effect of DMI [$F_{(2, 66)} = 21.83$, $p < 0.0001$] and interaction [$F_{(2, 66)} = 4.82$, $p = 0.0111$], but not of RIS

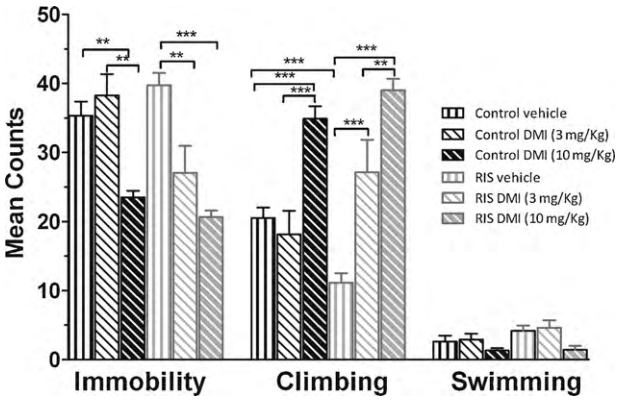

Fig. 2. Effects of chronic DMI administration on behavioural activity in the FST. Two days after the end of experimental protocol 2, all rats were subjected to the 5-min test of forced swim. The data corresponds to the mean ± SEM counts of immobility, climbing and swimming obtained from 22 (control vehicle), 6 (control DMI 3 mg/kg), 8 (control DMI 10 mg/kg), 22 (RIS vehicle), 6 (RIS DMI 3 mg/kg) and 8 (RIS DMI 10 mg/kg) independent rats, respectively. A two-way ANOVA showed significant effects of DMI on immobility and climbing, but not on swimming behaviour. The *post hoc* test gave the following significance between groups: $^{**}p < 0.01$, $^{***}p < 0.001$.

[$F_{(2, 66)}$ = 2,144, p = 0.148] on immobility scores, indicating that RIS does not influence immobility, and that the effect of DMI is different on control rats compared to repeated stressed rats. DMI administration at both doses studied (3 and 10 mg/kg) significantly increased climbing behaviour in repeated stressed rats. The two-way ANOVA showed a significant effect of DMI [$F_{(2, 64)}$ = 52.08, p < 0.0001] and interaction [$F_{(2, 64)}$ = 10.30, p = 0.0001], but not of RIS[$F_{(2, 64)}$ = 0.193, p = 0.661] on climbing behaviour. Furthermore, treatment with 10 mg/kg of DMI, but not of 3 mg/kg, significantly increased climbing behaviour in control rats. Thus, treatment with the lower dose of DMI fully prevents the decrease of climbing behaviour induced by RIS, without affecting this behavioural response in control rats. There was no effect of DMI treatment and RIS on swimming behaviour, but there is a significant interaction [$F_{(2, 65)}$ = 3.311, p = 0.042] indicating that the effect of DMI is different on control and repeated stressed rats. The treatment with the 10 mg/kg of DMI decreases the body weight gain and the DMI treatments did not prevent the increase of adrenal weight induced by RIS (supplemental Fig. 1 and Table 1).

3.3. Effect of subchronic DMI administration on the active behaviours in the FST

One day after the end of experimental protocol 3, rats of each experimental group were subjected to the FST pretest followed by subchronic administration of DMI (10 mg/kg) or vehicle at 23.5, 5 and 1 h before the 5-min test session. This subchronic DMI administration significantly increased climbing scores in both, control rats and in rats subjected to RIS (Fig. 3). Interestingly, this subchronic DMI administration exerted a greater effect in the climbing behaviour of repeatedly stressed rats than their respective controls (Fig. 3). The two-way ANOVA showed a significant effect of DMI [$F_{(1, 22)}$ = 31.20, p < 0.0001] and interaction [$F_{(1, 22)}$ = 7.03, p = 0.014], but not of RIS, indicating that the effect of DMI is different on control rats compared to repeatedly stressed rats. The subchronic DMI administration significantly decreased immobility in both control rats and in rats subjected to RIS (Fig. 3). The two-way ANOVA analysis showed a significant effect of subchronic DMI on immobility score ($F_{(1, 22)}$ = 40.81, p < 0.0001). No significant effects of RIS and DMI treatment were observed on swimming behaviour. However, a significant interaction [$F_{(1, 22)}$ = 10.19, p = 0.0042] was observed indicating that the effect of the subchronic DMI is different on control rats compared to repeatedly stressed rats.

3.4. Effects of DMI treatments on locomotor activity

One day after the end of experimental protocol 2 or 3, and before the FST, rats of each experimental group were subjected to the locomotor activity test. Chronic DMI treatment with 10 but not 3 mg/kg significantly decreased locomotor activity in both control rats and in rats subjected to RIS. The two-way ANOVA analysis showed a significant effect of chronic DMI treatment [$F_{(2, 54)}$ = 4.42, p = 0.0167], but not of RIS and interaction. Subchronic DMI significantly decreased locomotor activity (Table 1). The two-way ANOVA analysis showed a significant effect of subchronic DMI [$F_{(1, 47)}$ = 26.23, p < 0.0001], but not of RIS and interaction.

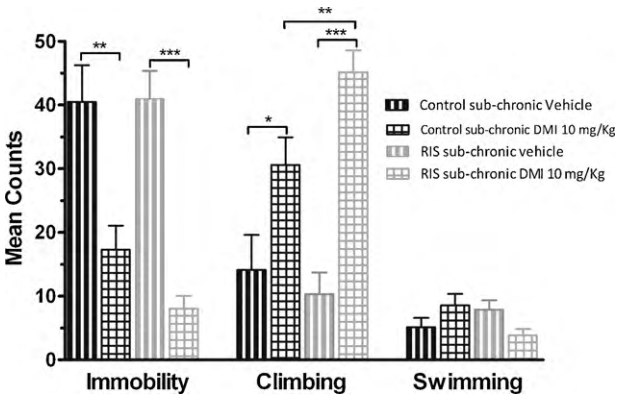

Fig. 3. Effects of subchronic DMI administration on behaviour activity in the FST. Two days after the end of experimental protocol 3, all rats were subjected to the 5-min test of forced swim. The data corresponds to the mean ± SEM counts of immobility, climbing and swimming obtained from 6 (control subchronic vehicle), 7 (control subchronic DMI 10 mg/kg), 7 (RIS subchronic vehicle), and 6 (RIS subchronic DMI 10 mg/kg) independent rats, respectively. A two-way ANOVA showed effects of DMI on immobility and climbing, but not on swimming behaviour. The analysis also showed a significant effect of RIS and interaction in climbing behaviour. The *post hoc* test gave the following significance between groups: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

4. Discussion

The main finding of our work is that repeated stress by immobilization decreased the baseline of climbing behaviour in the FST, while increased this behaviour in response to the subchronic administration of DMI. The results also show that the concomitant administration of the low dose of DMI (3 mg/Kg) during the RIS fully prevented the decrease of climbing behaviour induced by RIS, without exerting behavioural effects in control rats, further supporting an augmented response to DMI antidepressant effects in the repeatedly stressed rats. However, the administration of the high dose of DMI (10 mg/Kg) during 15 days increased climbing and decreased immobility in the FST in both control and repeatedly stressed rats, suggesting that at this dose DMI increases climbing *per se*. Taken together, our data indicate that RIS not only changes the behavioural responses in the FST but also increases the antidepressant effects of DMI. Finally, we suggest that DMI exerts its antidepressant effect by opposing the change induced by repeated stress.

The present results show that RIS induced a depressive-like behaviour as assessed by a decrease in the baseline of climbing in FST. This result is in agreement with previous findings showing

Table 1
Effect of DMI on locomotor activity.

Condition	Crosses DMI 3 mg/kg	*N*	Crosses DMI 10 mg/kg	*N*	Crosses DMI subchronic 10 mg/kg	*N*
Control vehicle	28.6 ± 3.0	14	28.6 ± 3.0	14	24.7 ± 4.3	12
Control DMI	27.5 ± 4.4	6	20.0 ± 3.3	10	10.3 ± 2.3**	14
RIS vehicle	32.0 ± 4.2	21	32.0 ± 4.2	21	26.9 ± 2.1	13
RIS DMI	27.7 ± 4.6	6	20.2 ± 2.5*	9	10.8 ± 3.0***	12

The values are mean ± SEM in a 30 min session of locomotor activity. Rats were administered with DMI (3 or 10 mg/kg) during 15 days, or three 10 mg/kg doses at 24.5, 5 and 1 h previous to the swim test (DMI subchronic). A two-way ANOVA analysis showed a significant effect of all DMI treatments. The *post hoc* test showed significant differences between groups.

* $p < 0.05$ RIS DMI 10 mg/kg compared to RIS vehicle.
** $p < 0.01$ control subchronic DMI compared to control vehicle.
*** $p < 0.001$ RIS subchronic DMI compared to RIS subchronic DMI.

that chronic unpredictable stress also decreases struggling (climbing) time in Sprague–Dawley rats (S–D) [29]. Tejani-Butt et al. [29] also showed that this stress effect was significantly higher in Wistar Kyoto rats (WKY) considered as an animal model of depressive behaviour, because they are prone to adopt passive-coping strategies in response to stress [22,29]. Moreover, the behavioural response of WKY control rats was similar to the stressed S–D rats, suggesting that chronic stress changes the behavioural effects of the forced swimming. Interestingly, the present results also showed a significantly greater increase of climbing behaviour in FST in response to the subchronic administration of DMI, a NRI antidepressant, in repeatedly stressed rats. This result suggests that RIS induced an augmented sensitivity to the antidepressant effects of DMI. In this regard, previous studies have shown that a lower dose of DMI was needed to decrease immobility and increase active behaviours in WKY compared to S–D rats, also suggesting an augmented sensitivity to the antidepressant effects of DMI [17]. Moreover, these two strains also show differences in baseline FST behaviour, the WKY rats show an increased immobility and a decreased climbing behaviour compared with S–D rats [17].

The decreased baseline of climbing in the FST and the augmented climbing response to the subchronic administration of DMI observed in repeatedly stressed rats could be accounted by increased noradrenergic activity accompanied with enhanced NA reuptake. In agreement with this possibility, it has been shown that immobilization stress increases the synthesis and turnover of NA in the PVN and CeA. Pacák et al. [19,20] have shown that a single exposure (2 h) of immobilization stress increases the synthesis, release and reuptake of NA in the PVN and in CeA. They also showed a higher increase of NA release during immobilization in repeatedly stressed rats (2 h/day for 7 days) in the PVN, but not in the CeA, compared to singly stressed rats. Moreover, after 6 days of RIS the NA basal levels were decreased, while the deaminated metabolite of NA was unchanged suggesting an enhanced NA reuptake in the PVN [19,20]. An enhanced reuptake of NA, possibly mediated by an increase in NA transporter (NAT) expression, induced by repeated stress may lead to decreased extracellular levels of NA in the brain regions underlying climbing behaviour expression. This decrease in extracellular levels of NA could explain the decreased baseline of climbing and the augmented climbing in response to subchronic DMI administration. In support of this hypothesis, Tejani-Butt et al. [29] showed that WKY rats had a significantly higher NAT density in the hippocampus and amygdala than S–D control rats. They also showed that chronic unpredictable stress significantly increases NAT density in the hippocampus and hypothalamus of S–D rats, suggesting that changes in the expression of NAT may underlie depressive-like behaviour induced by chronic stress. In the same line, it has been shown that chronic cold stress also increases NAT expression in the prefrontal cortex [18]. Thus, chronic exposure to different physical and emotional stressors induces an increase in NAT expression.

Consistent with an augmented response to the antidepressant effects of DMI induced by RIS, the concomitant treatment with 3 mg/kg DMI during RIS increases climbing behaviour in repeatedly stressed rats compared to vehicle treated stressed rats. Thus, this chronic DMI treatment prevented the decrease in baseline of climbing behaviour score. This effect was observed 2 days after the last administration of this low DMI dose, when acute DMI effects should be absent [35]. The antidepressant effect of the low dose of DMI in the FST was evident in repeatedly stressed rats, but not in naïve control rats, further supporting a role of the noradrenergic reuptake system on both the behavioural changes induced by RIS and the antidepressant effect of DMI. In agreement, previous evidence has shown that in WKY rats, an animal model of depressive behaviour, the chronic administration of DMI decreased immobility and increased active behaviour without inducing antidepressant effects in Wistar and S–D rats [30], further supporting that the antidepressant effect of DMI is augmented in pathological conditions. On other hand, treatment with the high dose of DMI (10 mg/kg) induces an antidepressant-like effect in the FST on both control and repeatedly stressed rats. Thus, besides preventing the depressive-like effect induced by RIS, this high dose of DMI enhances active-copping behaviour in control rats, probably by increasing NA extracellular levels. These results are in agreement with previous findings showing that rats treated during 14 days with high doses of DMI (10 or 15 mg/kg), but not with a low dose (5 mg/kg), decrease NAT expression in cerebral cortex and hippocampus [35]. Treatment with 15 mg/kg of DMI increases NA basal levels in the prefrontal cortex and decreases immobility [35]. Both effects were observed 2 days after ending DMI treatment, when DMI plasma and brain levels were undetectable [35]. Furthermore, the administration of a tyrosine hydroxylase inhibitor increases immobility on *naïve* rats and prevents the antidepressant effect in rats chronically treated with DMI [35]. Indeed, it has been shown that chronic treatment with NRI's or SSRI's, decreases NAT and SERT binding sites and expression, respectively in a time and dose dependent fashion [3,13,14,35,36].

In conclusion, our results show that RIS induces a noradrenergic-dependent depressive-like behaviour and an augmented response to the antidepressant effects of DMI. It is tempting to suggest that DMI exerts its antidepressant effect acutely by blocking NAT and chronically by decreasing NAT expression, counteracting the increase in NAT induced by repeated stress. The available data and present results let us to propose that RIS is a good model to study mechanisms underlying noradrenergic-dependent depression [6,10]. The observed behavioural effects of DMI *per se* may have clinical implications that should be further addressed.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbr.2010.05.037.

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