Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 520 (2005) 100–107

www.elsevier.com/locate/ejphar

Stressors affect the response of male and female rats to clomipramine in a model of behavioral despair (forced swim test)

Daniele Consoli [a], Julia Fedotova [b], Vincenzo Micale [a], Nikolay S. Sapronov [b], Filippo Drago [a,*]

[a] *Department of Experimental and Clinical Pharmacology, Faculty of Medicine, University of Catania Medical School, Viale A. Doria, 6 95125 Catania, Italy*
[b] *Department of Neuropharmacology, Institute of Experimental Medicine of the Russian Academy of Medical Sciences, St. Petersburg, Russia*

Received 19 May 2005; received in revised form 2 August 2005; accepted 8 August 2005
Available online 8 September 2005

Abstract

Aim of the present study was to evaluate the effects of physical stressors (electric foot-shocks) on effect of the antidepressant drug, clomipramine and plasma corticosterone levels in male and female rats tested in a model of behavioral despair (forced swim test,). Male and female rats of the Wistar strain were injected with clomipramine (50 mg/kg, i.p.) or saline. A group of animals also received electric shocks of different intensity and duration of 24, 5 and 1 h before being subjected to forced swim test. At the end of behavioral procedures, vaginal smears were assessed in all female animals and data on immobility time were plotted according to the ovarian cycle phase. After decapitation, corticosterone plasma levels were measured by radioimmunoassay in both male and female rats. Application of mild shocks (5 ms, 0.1 mA) significantly reduced immobility time in forced swim test of untreated male rats and augmented clomipramine effect on this parameter. Moderate shocks of higher intensity or duration (5 ms, 1.0 mA) also resulted in decreased immobility time of untreated male rats, but in reduced effect of clomipramine treatment. Furthermore, application of severe shocks (10 ms, 1.0 mA) increased the immobility time in untreated animals and totally abolished clomipramine effect in forced swim test. Untreated non-shocked female rats in proestrous and estrous phases exhibited a longer immobility time as compared to diestrous animals. Immobility time appeared to be generally higher when mild, moderate or severe shocks were applied prior to behavioral testing in proestrous and estrous animals, while the behavioral response of diestrous and metestrous animals did not differ from that of controls. Clomipramine effect on immobility time was generally reduced by application of shocks of every strengths. Stress-induced plasma corticosterone levels surge correlated with intensity and duration of shocks in both male and female rats, but clomipramine treatment generally blunted the hormonal response. However, severe shocks were followed by a surge of plasma corticosterone levels in both male and female clomipramine-treated rats.

These results demonstrate that duration and intensity of stressful stimuli may deeply affect the behavioral response of rats in forced swim test and influence clomipramine effect in this behavioral model depending on gender-based variables, probably of the hormonal type. Plasma corticosterone levels correlate with the behavioral response to clomipramine treatment suggesting that reactivity of hypothalamus–pituitary–adrenal axis to stress may be involved in the antidepressant effect of this drug.

Keywords: Stressor; Electric foot-shock; Corticosterone; Clomipramine; Depression; Gender; Forced swim test

1. Introduction

It is widely accepted that stress may be involved in the clinical manifestation of depression (Paykel, 1978; Checkley, 1992; Stout and Nemeroff, 1994; Mazure, 1995). Risk factors for depression can include, in fact, stressful life events in the period leading up to the depressive episode. Depressive people have frequently a great number of these events leading up to the psychic disorder (Andrews and Tennant, 1978; Faravelli et al., 1986; Paykel, 2001; Tennant, 2002). Furthermore, it is well known that women are more susceptible to depression than men (Piccinelli and Wilkinson, 2000; Bogner and Gallo, 2004; Goodwin and Gotlib, 2004).

* Corresponding author. Tel.: +39 0957384236; fax: +39 0957384238.
E-mail address: fdrago@tin.it (F. Drago).

0014-2999/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2005.08.012

Despite of this clinical evidence, however, experimental models of depression are currently not taking care of the possible influence of pre-training stress on the depressive response of animals, and of the gender-based differences in this response. Besides, literature is very poor of studies on the influence of manipulation, drug injection procedure or other kind of psychic or physic stressors on the behavioral response of animals in experimental models of depression. For example, repeated saline injection may induce a depressive-like state in rats, which is counteracted by the antidepressant drug, imipramine (Izumi et al., 1997). Furthermore, subcutaneous saline injections per se may affect the response of rats (Drago et al., 2001) in a commonly used experimental model of behavioral despair, the forced swim test. Indeed, the efficacy of antidepressant drugs in experimental model of depression may be deeply influenced by application of different kinds of stressor prior to behavioral testing.

Stress can influence central nervous system (CNS) functions by complex neuro-hormonal changes. Among them, activation of the hypothalamus–pituitary–adrenal axis and catecholamines release from adrenal medulla are the fundamentals for the adaptive response (Weiss et al., 1970). It is also known that other hormonal mediators are involved in the response to stress (Sandman et al., 1973; Krulich et al., 1974; Brennan et al., 1975; Guillemin et al., 1977; Drago, 1990). The stress system orchestrates body and brain responses to the environment with two modes of operation, the first being involved in acute response to stressor and the second facilitating behavioral adaptation (De Kloet, 2003). Corticosteroid hormones are implicated in both modes through their high affinity mineralocorticoid receptors and lower affinity glucocorticoid receptors. Imbalance induced by chronic stress in the two system modes changes specific neural signaling pathways underlying psychic domains of behavior, including mood (De Kloet, 2003). Besides, changes of the hypothalamus–adrenal axis hormones have been described in depressed patients (Seckl et al., 1990; Gehris et al., 1991). The reduced negative feedback response to exogenous glucocorticoids is one of the most consistent findings, and is characterized by the failure in suppression of plasma cortisol levels following administration of synthetic glucocorticoid, dexamethason (Carroll et al., 1981; Kalin et al., 1982; Holsboer, 1983). Hypersecretion of basal cortisol (Carroll et al., 1976) and augmented adrenal weight (Rubin et al., 1995) are also observed.

In stress-related animal models of depression, antidepressant treatment not only reduces the depressive-like behavior (Porsolt et al., 1977) but also counteracts stress-induced hormonal changes. In particular, the antidepressant drug, clomipramine counteracts stress-induced surge of corticosterone plasma levels (Fuchs et al., 1996) and variation in CNS neurotransmitter metabolites (Van der Hart et al., 2002). However, it is not known whether and in which extent stressors of different strength might influence the response of animals and the effect of antidepressant drugs in experimental models of depression.

Based on the above rationale, the present study was performed for evaluating the influence of different strength stressors on clomipramine effect assessed in forced swim test and its possible correlation with corticosterone response in both male and female rats. Among various experimental models, the forced swim test was selected as judged to highly fulfill face, construct and predictive criteria established for experimental models of mental diseases and is widely used for pre-clinical studies on novel antidepressant drugs (Porsolt et al., 1977; Willner, 1984). In order to obtain a precise evaluation of stress influence on behavioral performance, variable electrical shocks with different intensity and duration were used. Acute shock paradigm followed by forced swim test was selected in order to distinguish the stress procedure from the experimental model of behavioral despair. In this manner, we could modify shock variables (duration and/or intensity) without affecting the behavioral procedure. In fact, a chronic shock paradigm such as chronic mild stress affects per se the response of animals to antidepressants and is considered as an experimental model of depression. Corticosterone plasma levels were measured to evaluate a possible correlation between stressor strength and hormonal response under clomipramine treatment. Experiments were made in both male and female rats to provide a wider idea on the possible clinical extension of these results evaluating the gender influence on the correlation between clomipramine effect and corticosterone response to stress.

2. Materials and methods

2.1. Animals

Male rats of the Wistar strain (purchased from Charles River, Italy) weighing 220–240 g were used. Female rats of the same strain (weighing 200–220 g), purchased from the same provider, were also used. For at least a week prior to the experiments, the rats were housed four to a cage under standard environmental conditions: constant temperature of 23 ± 1 °C, 60% humidity, 12-h light/dark cycle (lights on between 8.00 and 20.00), food and water available ad libitum. The care and maintenance of the rats conformed to the European Communities Council Directive 86/609/EEC and efforts were made to minimize animal suffering and to reduce the number of animals used. The rationale, design and methods of this study have been approved by the Ethical Committee for Animal Research, University of Catania.

2.2. Vaginal smears

After a week of habituation in the facilities, female rats were subjected daily to vaginal smears. Vaginal samples were taken flushing 60 to 75 μl of methylene blue (0.1% in water) in and out of the vagina using a micropipette and then placing a drop of exudate on a slide. The resulting

cytological display of exfoliated cells showed histological variations that indicated under light microscopy the ovarian phase as diestrous, metestrous, proestrous, and estrous (cornified cells with acidophilic cytoplasm), based on the predominant cell type.

2.3. Drugs and treatment

Clomipramine hydrochloride, purchased from Sigma (USA), was freshly diluted in physiological saline and injected intraperitoneally (i.p.). Rats received three such injections 24, 5 and 1 h prior to behavioral testing. Physiological saline as placebo was injected i.p. to control animals with the same procedure.

All animals were gently handled each day for a week prior to experimental procedures by experienced facilities' keepers avoiding any environmental or physic stress in order to make them habituated to manipulation. Animals were randomly assigned to any treatment group and were used only once in the behavioral experiments. Rats subjected to drug or placebo administration received an injection of a 1-ml standard volume of solution with a 23-gauge stainless steel needle of 31 mm length.

2.4. Forced swim test

Rats were individually forced to swim inside vertical plexiglas cylinders containing 15 cm of water maintained at 25 °C (Porsolt et al., 1977). After 15 min in the water, they were removed and allowed to dry for 15 min in a heated container before being returned to their home cages. They were replaced in the cylinders 24 h later and total duration of immobility (immobility time) was measured during a 5-min test. A rat was judged to be immobile whenever it remained passively floating in the water in a slightly hunched but upright position, its head just above the surface.

2.5. Plasma corticosterone measurement

After behavioral procedures were completed and vaginal smears were made in female rats, animals were immediately killed by decapitation. The blood samples were collected and centrifuged, and the plasma was frozen at −20 °C until assayed for corticosterone concentrations by radioimmunoassay. The [^{125}I]-labeled corticosterone (48.1 kBq) double antibody radioimmunoassay kit for rats (Amersham, USA) was used. To displace corticosterone from corticosterone-binding globulin in plasma, the plasma was heated for 30 min at 60 °C. The assay was carried out at room temperature, using rabbit anti-corticosterone serum as the first antibody and donkey anti-rabbit serum coated on magnetizable polymer particles as the second antibody. According to the manufacturer, the cross reactivity is very low. The highest cross-reactivity is found with 11-deoxycorticosterone (2.4% in contrast to 100% for corticosterone). Plasma corticosterone levels were expressed as μg/100 ml.

2.6. Experimental design

In a preliminary experiment the dose–effect relationship of clomipramine compared with placebo was studied in forced swim test. This experiment was carried out to verify test suitability in our experimental plan. For this reason we selected three doses of clomipramine (1, 10, 50 mg/kg) and found the 50 mg/kg dose being fully active in reducing the immobility time. This dose was selected for the succeeding experiments. Furthermore, this dose was considered as the minimum effective dose of clomipramine in forced swim test (Porsolt et al., 1977).

Two different experiments were programmed and carried out. In the first experiment, the effect of electric shocks on the response of male rats in forced swim test was studied. For this purpose, groups of 7 animals were injected i.p. with clomipramine or saline and subjected to electric shocks 24, 5 and 1 h prior to behavioral testing. This treatment schedule

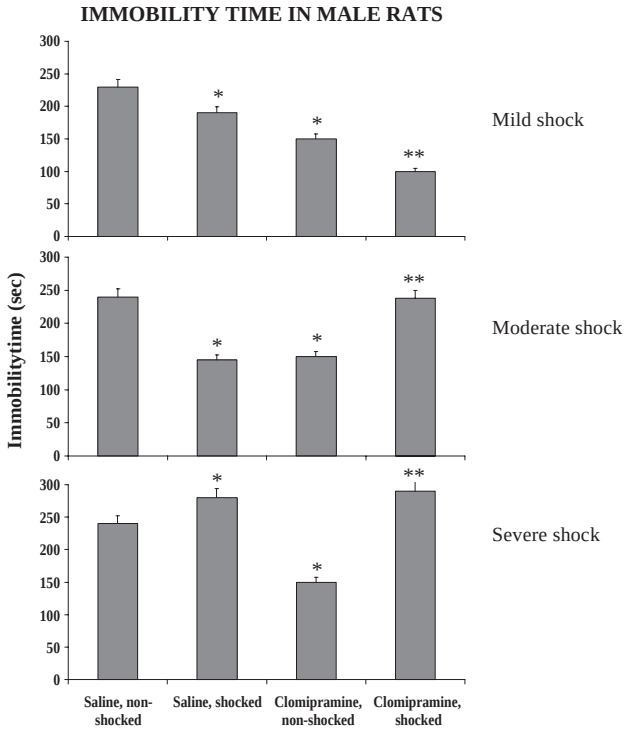

Fig. 1. Effects of clomipramine treatment on the immobility time exhibited by male rats tested in forced swim test after application of stressors (electric foot-shocks) of various duration and intensity. * Significantly different as compared to saline-non-shocked controls ($P \leq 0.05$, Dunnett's test for multiple comparisons). ** Significantly different as compared to saline-non-shocked controls and to clomipramine-treated non-shocked animals ($P \leq 0.05$, Dunnett's test for multiple comparisons). Values are mean±SEM. The number of animals was 7 per group. Shocks were as follows: mild (5 ms, 0.1 mA), moderate (5 ms, 1.0 mA), severe (10 ms, 1.0 mA). Animals were injected i.p. with clomipramine (50 mg/kg) or saline and subjected to electric shocks 24, 5 and 1 h prior to behavioral testing. Immobility time was the total time of passive floating in the water in a 5-min test. Two-way ANOVA revealed a significant treatment–time interaction for mild [$F(3,27)=3.35$, $P<0.01$], moderate [$F(3,27)=3.21$, $P<0.01$] and severe shock [$F(3,27)=3.42$, $P<0.01$].

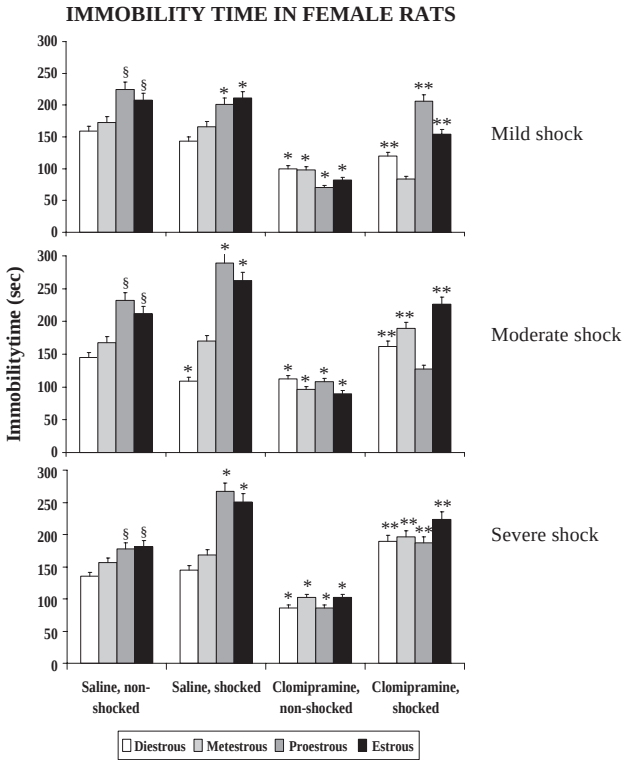

Fig. 2. Effects of clomipramine treatment on the immobility time exhibited by female rats in various phases of ovarian cycle tested in forced swim test after application of stressors (electric foot-shocks) of various duration and intensity. §Significantly different as compared to saline-non-shocked diestrous controls ($P \leq 0.05$, Dunnett's test for multiple comparisons). *Significantly different as compared to saline-non-shocked controls ($P \leq 0.05$, Dunnett's test for multiple comparisons). **Significantly different as compared to saline-non-shocked controls and to clomipramine-treated non-shocked animals ($P \leq 0.05$, Dunnett's test for multiple comparisons). Values are mean ± SEM. The number of animals was 7 per group of ovarian cycle phase. Shocks were as follows: mild (5 ms, 0.1 mA), moderate (5 ms, 1.0 mA), severe (10 ms, 1.0 mA). Animals were injected i.p. with clomipramine (50 mg/kg) or saline and subjected to electric shocks 24, 5 and 1 h prior to behavioral testing. Immobility time was the total time of passive floating in the water in a 5-min test. Two-way ANOVA revealed a significant treatment–time interaction for mild [$F(15,111)=4.95$, $P<0.05$], moderate [$F(15,111)=4.45$ $P<0.05$] and severe shock [$F(15,111)=4.81$, $P<0.05$].

was adopted in all experiments in accordance to the original procedure described by Porsolt et al. (1977). Application of electric shocks was made just after each injection of clomipramine or saline in order to ensure specific and constant treatment/stressor interaction. Duration of shocks was 5 or 10 ms and intensity was 0.1 or 1 mA. Rats received three such shocks through an electric grid put in a box with transparent walls. A group of non-shocked and untreated animals were considered as controls. At the end of behavioral procedure, all animals were sacrificed by decapitation and corticosterone plasma levels were measured.

In the second set of experiments, groups of 7 female rats were selected according to phase of their ovarian cycle (as assessed by vaginal smears) and injected i.p. with clomipramine or saline with the same procedure used for male animals. They were subjected to electric shocks 24, 5 and 1 h prior to behavioral testing. Application of electric shocks was made just after each injection of clomipramine or saline. Duration of shocks was 5 or 10 ms and intensity was 0.1 or 1 mA. A group of non-shocked and untreated animals were considered as controls. All animals were sacrificed by decapitation and corticosterone plasma levels were measured with the same procedure used for male animals.

2.7. Statistical analysis

All data were analyzed using two-way analysis of variance (two-way ANOVA) and the post hoc Dunnett's test for multiple comparisons. A P-value of 0.05 or less was considered as indicative of a significant difference. Where not indicated, two-way ANOVA revealed no significant level of variance.

3. Results

Application of mild or moderate shocks (5 ms, 0.1 or 1 mA) reduced immobility time of untreated male rats as compared to saline-injected non-shocked controls (Fig. 1). In contrast, severe shocks (10 ms, 1 mA) caused a sustained increase in immobility time of untreated male rats. Clomipramine treatment was followed by similar reduced immobility time in all three groups of non-shocked animals as compared to that of untreated non-shocked controls. Furthermore, animals treated with clomipramine and subjected to mild shocks exhibited an immobility time lower than that of clomipramine-treated non-shocked animals. In

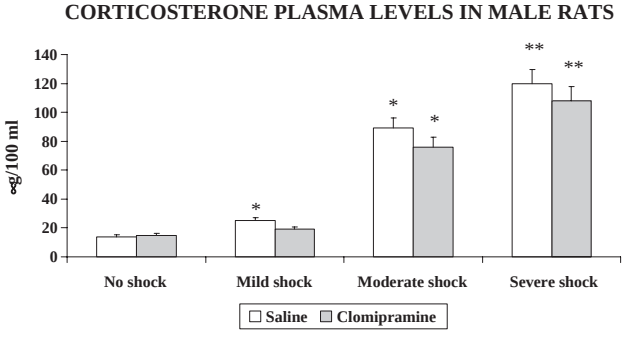

Fig. 3. Effects of clomipramine treatment on plasma corticosterone levels in male rats tested in forced swim test after application of stressors (electric foot-shocks) of various duration and intensity. *Significantly different as compared to saline-non-shocked controls ($P \leq 0.05$, Dunnett's test for multiple comparisons). **Significantly different as compared to saline-non-shocked controls and to saline-injected or clomipramine-injected animals subjected to mild or moderate shocks ($P \leq 0.05$, Dunnett's test for multiple comparisons). Values are mean ± SEM. The number of animals was 7 per group. Shocks were as follows: mild (5 ms, 0.1 mA), moderate (5 ms, 1.0 mA), severe (10 ms, 1.0 mA). Animals were injected i.p. with clomipramine (50 mg/kg) or saline and subjected to electric shocks 24, 5 and 1 h prior to behavioral testing. After behavioral procedures were completed, animals were immediately killed by decapitation and blood samples were withdrawn. Two-way ANOVA revealed a significant treatment–time interaction [$F(6,55)=3.35$, $P<0.01$].

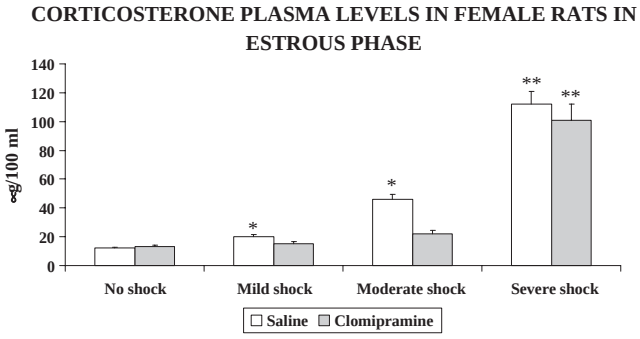

Fig. 4. Effects of clomipramine treatment on plasma corticosterone levels in female rats in estrous phase of ovarian cycle tested in forced swim test after application of stressors (electric foot-shocks) of various duration and intensity. * Significantly different as compared to saline-non-shocked controls ($P \leq 0.05$, Dunnett's test for multiple comparisons). ** Significantly different as compared to saline-non-shocked controls and to saline-injected or clomipramine-injected animals subjected to mild or moderate shocks ($P \leq 0.05$, Dunnett's test for multiple comparisons). Values are mean ± SEM. The number of animals was 7 per group. Shocks were as follows: mild (5 ms, 0.1 mA), moderate (5 ms, 1.0 mA), severe (10 ms, 1.0 mA). Animals were injected i.p. with clomipramine (50 mg/kg) or saline and subjected to electric shocks 24, 5 and 1 h prior to behavioral testing. After behavioral procedures were completed, animals were immediately killed by decapitation and blood samples were withdrawn. Two-way ANOVA revealed a significant treatment–time interaction. Two-way ANOVA revealed a significant treatment–time interaction [$F(6, 55) = 3.67$, $P < 0.01$].

contrast to mild shocks, however, moderate and severe shocks caused a reduction in clomipramine effect. In fact, immobility time of rats treated with clomipramine and subjected to moderate or severe shocks was found to be higher than that of untreated or clomipramine-treated non-shocked animals. In particular, severe shocks totally abolished clomipramine effect in reducing immobility time in forced swim test.

In female rats, phases of ovarian cycle appeared to affect immobility time in forced swim test. All groups of saline-injected non-shocked animals in proestrous and estrous phases exhibited an immobility time longer than those in diestrous phase (Fig. 2). Application of mild, moderate or severe shocks prior to behavioral testing induced an altered response in female rats depending on the ovarian phase. In particular, proestrous and estrous rats showed an increased immobility time, while the behavioral response of diestrous and metestrous animals did not differ from that of saline-injected non-shocked controls. The only exception was represented by diestrous rats subjected to moderate shocks that exhibited an immobility time in forced swim test lower than that of corresponding controls. Immobility time appeared to be decreased in clomipramine-treated non-shocked animals of all ovarian cycle phases as compared to that of untreated non-shocked controls. Application of mild, moderate or severe shocks was followed by increased immobility time in clomipramine-treated rats of all ovarian phases as compared to drug-treated non-shocked animals. Only clomipramine-treated metestrous and proestrous animals subjected to mild and moderate shocks, respectively, failed to exhibit significant changes in immobility time.

The measurement of plasma corticosterone levels revealed a correlation between strength (intensity and/or duration) of shocks and hormonal response in male rats (Fig. 3). However, animals treated with clomipramine and subjected to mild shocks failed to exhibit any significant change in plasma corticosterone levels. In fact, clomipramine treatment prevented stress-induced plasma corticosterone levels surge in these animals, but not in those subjected to moderate or severe shocks.

Female rats in estrous phase also exhibited increased hormonal response that correlated with shock strength (Fig. 4). In these animals, clomipramine treatment prevented corticosterone surge induced by mild or moderate shocks, but not by severe shocks. Like estrous rats, proestrous, diestrous or metestrous animals showed increased plasma corticosterone levels that correlated with shock strength. However, no change in corticosterone surge was found after clomipramine treatment (data not shown).

Finally, moderate shocks application was followed by more sustained increase of corticosterone levels in males than in female rats ($p < 0.05$, Dunnett's test for multiple comparisons).

4. Discussion

One result of this study's deserving attention is that low intensity physical stressors seem to exert antidepressant-like effects in male rats studied in the forced swim test. This is a finding consistent with the concept that mild acute stressors may stimulate brain functions in different experimental and clinical situations. Uncontrollable mild shocks have been found to induce behavioral invigoration in mice subjected to forced swim test and the controllability of the stressor does not influence the initial invigoration (Prince and Anisman, 1984). Furthermore, facilitation of cognitive processes has been described after administration of stress hormones, such as adrenocorticotropin hormone (ACTH) (Weiss et al., 1970; De Wied, 1979), vasopressin (Van Wimersma Greidanus et al., 1974, 1975), prolactin (Drago, 1990) and other pituitary hormones. Glucocorticoids may also facilitate storage of new information, but they facilitate the extinction of behavior that is no more relevant to the subject (De Kloet, 2003; Hui et al., 2004). In female rats, however, mild shocks did not result in anti-depressant effects in forced swim test and only diestrous animals subjected to moderate shocks exhibited decreased immobility time. Thus, gender-dependent hormonal milieu may influence the behavioral response of animals subjected to mild or moderate stressors.

Furthermore, clomipramine effect here appeared to be enhanced by application of mild shocks in male but not in female rats, suggesting the existence of gender-based variability in stress-induced variations of clomipramine effect. In a way, this finding agrees with the different

attitude to depressive behavior found in males compared to females tested in forced swim test.

It is generally accepted that corticosterone plasma levels are correlated to the duration and intensity of stressors. However, behavioral procedure per se might have contributed to the hypersecretion of corticosterone. In fact, Wistar Kyoto rats that display increased behavioral immobility also demonstrates exaggerated secretion of stress hormones during forced swim test, and the results may be due, in part, to reduced sensitivity of glucocorticoid receptors that supply negative feedback to the hypothalamus–pituitary–adrenal axis (Rittenhouse et al., 2002). As application of stressors in the present study was made three times along 24 h prior to behavioral testing, no adaptive response of this axis has been found. In fact, animals subjected to chronic stressors do not show plasma corticosterone levels higher than those of unstressed controls (Hilakivi-Clarke et al., 1992; Azpiroz et al., 1999).

Furthermore, clomipramine administration was followed by a reduced surge of plasma corticosterone after application of mild or moderate shocks. Tricyclic antidepressants appear to mitigate the multiple stress-induced neuroendocrine feedback loops forming the vicious circle to activate central corticotropin-releasing hormone (CRH) neuronal systems. (Makino et al., 2002). Indeed, antidepressant drugs counteract the reduction of locomotor activity and of catecholamine utilization in the brain induced by repeated stress (Zebrowska-Lupina et al., 1990). In particular, clomipramine counteracts stress-induced surge of corticosterone plasma levels (Fuchs et al., 1996) and changes in cerebral metabolites (Van der Hart et al., 2002). These findings are consistent with the clinical evidence of increased adrenal cortisol responses and baseline hypersecretion of cortisol in depressed patients (Sachar et al., 1973; Holsboer, 1983; Musselman and Nemeroff, 1995). In these patients, dysregulations of hypothalamus–pituitary–adrenal axis and of sympathetic system occur together, and increased cortisol levels are combined with a hypersecretion of norepinephrine (Musselman and Nemeroff, 1995). Like in depressed patients (Holsboer et al., 1983; Golden et al., 1988), treatment of rats with clomipramine normalizes both behavioral responses in forced swim test and activity of the hypothalamus–pituitary–adrenal axis. This may be due to direct interactions of the drug with serotonergic and/or noradrenergic circuits in various brain areas, which in turn, modulate the activity of the CRH system. This system stimulates the synthesis and release of ACTH and other pro-opiomelanocortin products from the pituitary and regulates autonomic activity (Tilders and Berkenbosch, 1986; Owens and Nemeroff, 1991). Interestingly, reduced brain serotonin levels have been found in an experimental model of depression after application of different kinds of stressor (Connor et al., 1999). However, when severe shocks were applied, both male and female rats showed increased plasma corticosterone levels. It is possible that failure of clomipramine in inhibiting flow of corticosterone under application of moderate or severe shocks in male rats is the neuroendocrine expression of drug inefficacy in behavioral measures.

Interestingly, moderate shocks application was followed by plasma corticosterone levels higher in male than in female rats. Under stress conditions, females may exhibit either higher (Wilson et al., 2004) or similar levels of adrenal hormones compared to males (Conrad et al., 2004; Drossopoulou et al., 2004). Furthermore, it should be noted that in most studies with female rats no correlation is made between stress-induced corticosterone surge and phase of ovarian cycle. Thus, another important issue related to the present findings is whether corticosterone response to stress in female animals depended on the ovarian phase. Although the behavioral response of female rats in forced swim test varied depending on the ovarian phase, only estrous animals exhibited a reduced surge of corticosterone after treatment with clomipramine. In other studies, plasma corticosterone levels vary in estrous animals subjected to different kinds of stressor (Rubinow et al., 2004) and females in proestrous showed higher plasma corticosterone levels during restraint stress compared to females in estrous (Conrad et al., 2004). As proestrous and estrous phases are associated to high plasma estrogen levels, it is possible that this factor influences the corticosterone response to stress.

Another interesting finding was that saline-injected non-shocked female rats in proestrous or estrous phase exhibited a prolonged duration of immobility time than animals in diestrous or metestrous phases. Since proestrous and estrous phases correspond with a surge of estrogens in female rats, while diestrous with lowest levels of estrogens, it is conceivable that a surge of these hormones may induce a depressive-like status in female rats. This concept is consistent with the finding that clomipramine reduced plasma corticosterone levels after application of mild or moderate shocks only in estrous female rats, while it failed to exhibit this effect in proestrous, diestrous and metestrous females. Furthermore, whenever they were subjected to shocks or clomipramine treatment, differences were observed in forced swim test response among females of various ovarian cycle phases. Results showed that proestrous and estrous females were more susceptible to moderate or severe shocks than diestrous animals (Fig. 2). Thus, hormonal changes related to ovarian cycle might have influenced their responsiveness to stress.

The clinical extension of this finding may be relevant for the pharmacological treatment of depression. In fact, the gender difference in prevalence and incidence rates of depression is one of the most consistent findings in psychiatric epidemiology (Piccinelli and Wilkinson, 2000; Bogner and Gallo, 2004; Goodwin and Gotlib, 2004) and rates of depression in women are approximately twice those seen in men (Cohen, 2003). Furthermore, higher incidence of depression has been reported for contraceptive users (Shaarawy et al., 1982). In contrast, low plasma estrogen levels in post-menopausal women seem to be associated with higher incidence of depression, although the efficacy of

estrogen replacement therapy in this pathology is uncertain (Carranza-Lira and Valentino-Figueroa, 1999; Morrison et al., 2004).

We should consider that different kinds of stressors have been shown to modify the behavioral response of animals tested in forced swim test. The simple repeated saline injection may induce a depressive-like state in rats that is counteracted by the antidepressant drug, imipramine (Izumi et al., 1997). More recently, we have shown that placebo injection per se may represent a stressor capable of inducing an increased immobility time in comparison to that found in intact non-injected controls (Drago et al., 2001). One direct consequence of these findings is that the net difference between placebo-injected and intact controls should be deducted in the evaluation of the pharmacological potency of antidepressant drugs studied with this test. However, the experimental design of the present study did not allow evaluating the effect of this stressor (injection per se) on clomipramine efficacy, as the drug could not be administered in a stressor-free situation.

A limiting factor in this study is the fact that a single antidepressant at single dose was evaluated. However, it should be considered that clomipramine is among the most studied antidepressants in pre-clinical studies and using multiple drug and dose schedule should have made much more complicated the experimental procedure of the present study.

In conclusion, a direct consequence of the present results is that correct experimental design of animal studies with the forced swim test should avoid any type of incontrollable stressors that may influence the efficacy of an antidepressant treatment to the animals. We have already proposed that correct experimental design with forced swim test should include two control groups, the placebo-treated and the untreated (unstressed) intact controls. An example of this design is shown in a previous study (Drago et al., 2001). Furthermore, the finding that severe stressful stimuli may facilitate a depressive response in male and female rats tested in forced swim test and diminish clomipramine efficacy deserves more attention for the possible correlates at the clinical level.

References

Andrews, G., Tennant, C., 1978. Life event stress and psychiatric illness. Psychol. Med. 8, 545–549.

Azpiroz, A., Fano, E., Garmendia, L., Arregi, A., Cacho, R., Beitia, G., Brain, P.F., 1999. Effects of chronic mild stress (CMS) and imipramine administration, on spleen mononuclear cell proliferative response, serum corticosterone level and brain norepinephrine content in male mice. Psychoneuroendocrinology 24, 345–361.

Bogner, H.R., Gallo, J.J., 2004. Are higher rates of depression in women accounted for by differential symptom reporting? Soc. Psychiatry Psychiatr. Epidemiol. 39, 126–132.

Brennan, T.C., Shelton, R.L., Robertson, G.L., 1975. Effect of stress on plasma ADH and corticosterone in rats. Clin. Res. 23, 234–239.

Carranza-Lira, S., Valentino-Figueroa, M.L., 1999. Estrogen therapy for depression in postmenopausal women. Int. J. Gynaecol. Obstet. 65, 35–38.

Carroll, B.J., Curtis, G.C., Mendels, J., 1976. Cerebrospinal fluid and plasma free cortisol concentrations in depression. Psychol. Med. 6, 235–244.

Carroll, B.J., Feinberg, M., Greden, J.F., Tarika, J., Albala, A.A., Haskett, R. F., James, N.M., Kronfol, Z., Lohr, N., Steiner, M., de Vigne, J.P., Young, E., 1981. A specific laboratory test for the diagnosis of melancholia: standardization, validation and clinical utility. Arch. Gen. Psychiatry 38, 15–22.

Checkley, S., 1992. Neuroendocrine mechanisms and the precipitation of depression by life events. Br. J. Psychiatr., Suppl. 15, 7–17.

Cohen, L.S., 2003. Gender-specific considerations in the treatment of mood disorders in women across the life cycle. J. Clin. Psychiatry 64 (Suppl 15), 18–29.

Connor, T.J., Song, C., Leonard, B.E., Anisman, H., Merali, Z., 1999. Stressor-induced alterations in serotonergic activity in an animal model of depression. NeuroReport 25, 523–528.

Conrad, C.D., Jackson, J.L., Wieczorek, L., Baran, S.E., Harman, J.S., Wright, R.L., Korol, D.L., 2004. Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle. Pharmacol. Biochem. Behav. 78, 569–579.

De Kloet, R.E., 2003. Hormones, brain and stress. Endocr. Regul. 37, 51–68.

De Wied, D., 1979. Neuropeptides and behavior. The neuropeptide concept. Ned. Tijdschr. Geneeskd. 123, 1589–1590.

Drago, F., 1990. Behavioral effects of prolactin. Curr. Top. Neuroendocr. 10, 263–289.

Drago, F., Nicolosi, A., Micale, V., Lo Menzo, G., 2001. Placebo affects the performance of rats in models of depression: is it a good control for behavioral experiments? Eur. Neuropsychopharmacol. 11, 209–213.

Drossopoulou, G., Antoniou, K., Kitraki, E., Papathanasiou, G., Papalexi, E., Dalla, C., Papadopoulou-Daifoti, Z., 2004. Sex differences in behavioral, neurochemical and neuroendocrine effects induced by the forced swim test in rats. Neuroscience 126, 849–857.

Faravelli, C., Sacchetti, E., Ambonetti, A., Conte, G., Pallanti, S., Vita, A., 1986. Early life events and affective disorder revisited. Br. J. Psychiatry 148, 288–295.

Fuchs, E., Kramer, M., Hermes, B., Netter, P., Hiemke, C., 1996. Psychosocial stress in tree shrews: clomipramine counteracts behavioral and endocrine changes. Pharmacol. Biochem. Behav. 54, 219–228.

Gehris, T.L., Kathol, R., Meller, W.H., Lopez, J.F., Jaeckle, R.S., 1991. Multiple steroid hormone levels in depressed patients and normal controls before and after exogenous ACTH. Psychoneuroendocrinology 16, 481–497.

Golden, R.N., Markey, S.P., Risby, E.D., Rudorfer, M.V., Cowdry, R.W., Potter, W.Z., 1988. Antidepressants reduce whole-body NE turnover while enhancing 6-hydroxymelatonin output. Arch. Gen. Psychiatry 45, 150–154.

Goodwin, R.D., Gotlib, I.H., 2004. Gender differences in depression: the role of personality factors. Psychiatry Res. 126, 135–142.

Guillemin, R., Vargo, T., Rossier, J., Minick, S., Ling, N., Rivier, J., Vale, W., Bloom, F., 1977. Beta endorphin and adrenocorticotropin are secreted concomitantly by the pituitary. Science 197, 1367–1369.

Hilakivi-Clarke, L.A., Arora, P.K., Sabol, M.B., Clarke, R., Dickson, R.B., Lippman, M.E., 1992. Alterations in behavior, steroid hormones and natural killer cell activity in male transgenic TGF_{α} mice. Brain Res. 588, 97–103.

Holsboer, F., 1983. The dexamethasone suppression test in depressed patients: clinical and biochemical aspects. J. Steroid Biochem. 19, 251–257.

Hui, G.K., Figueroa, I.R., Poytress, B.S., Roozendaal, B., McGaugh, J.L., Weinberger, N.M., 2004. Memory enhancement of classical fear conditioning by post-training injections of corticosterone in rats. Neurobiol. Learn. Mem. 81, 67–74.

Izumi, J., Washizuka, M., Hayashi-Kuwabara, Y., Yoshinaga, K., Tanaka, Y., Ikeda, Y., Kiuchi, Y., Oguchi, K., 1997. Evidence for a depressive-like state induced by repeated saline injections in Fischer 344 rats. Pharmacol. Biochem. Behav. 57, 883–888.

Kalin, N.H., Weiler, S.J., Shelton, S.E., 1982. Plasma ACTH and cortisol concentration before and after dexamethasone. Psychiatric. Res. 7, 87–92.

Krulich, L., Hefco, E., Illner, P., Read, C.B., 1974. The effects of acute stress on the secretion of LH, FSH; PRL and GH in the normal male rat, with comments on their statistical evaluation. Neuroendocrinology 16, 293–311.

Makino, S., Hashimoto, K., Gold, P.W., 2002. Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. Pharmacol. Biochem. Behav. 73, 147–158.

Mazure, C.M., 1995. Does stress cause psychiatric illness? In: Spiegel, D. (Ed.), Progress in Psychiatry. American Psychiatric, Washington, pp. 270–280.

Morrison, M.F., Kallan, M.J., Ten Have, T., Katz, I., Tweedy, K., Battistini, M., 2004. Lack of efficacy of estradiol for depression in postmenopausal women: a randomized, controlled trial. Biol. Psychiatry 55, 406–412.

Musselman, D.L., Nemeroff, C.B., 1995. Adrenal function in major depression. Endocrinologist 5, 91–96.

Owens, M.J., Nemeroff, C.B., 1991. Physiology and pharmacology of corticotropin-releasing hormone. Pharmacol. Rev. 43, 425–473.

Paykel, E.S., 1978. Contribution of life events to causation of psychiatric illness. Psychol. Med. 8, 245–253.

Paykel, E.S., 2001. Stress and affective disorder in rats: a new model sensitive to antidepressant treatments. Eur. J. Pharmacol. 47, 379–385.

Piccinelli, M., Wilkinson, G., 2000. Gender differences in depression. Critical review. Br. J. Psychiatry 177, 486–492.

Porsolt, R.D., Le Pichon, M., Jalfre, M., 1977. Depression: a new animal model sensitive to antidepressant treatment. Nature 266, 730–732.

Prince, C.R., Anisman, H., 1984. Acute and chronic stress effects on performance in a forced-swim task. Behav. Neural Biol. 42, 99–119.

Rittenhouse, P.A., Lopez-Rubalcava, C., Stanwood, G.D., Lucki, I., 2002. Amplified behavioral and endocrine responses to forced swim stress in the Wistar-Kyoto rat. Psychoneuroendocrinology 27, 303–318.

Rubin, R.T., Phillips, J.J., Sadow, T.F., McCracken, J.T., 1995. Adrenal gland volume in major depression. Increase during the depressive episode and decrease with successful treatment. Arch. Gen. Psychiatry 52, 213–218.

Rubinow, M.J., Arseneau, L.M., Beverly, J.L., Juraska, J.M., 2004. Effect of the estrous cycle on water maze acquisition depends on the temperature of the water. Behav. Neurosci. 118, 863–868.

Sachar, E.J., Hellman, L., Roffwarg, H.P., Halpern, F.S., Fukushima, D.K., Gallagher, T.F., 1973. Disrupted 24-hour patterns of cortisol secretion in psychotic depression. Arch. Gen. Psychiatry 28, 19–24.

Sandman, C.A., Kastin, A.J., Schally, A.V., Kendall, J.W., Miller, L.H., 1973. Neuroendocrine responses to physical and psycological stress. J. Comp. Physiol. Psychol. 84, 386–390.

Seckl, J.R., Campbell, J.C., Edwards, C.R., Christie, J.E., Whalley, L.J., Goodwin, G.M., Fink, G., 1990. Diurnal variation of plasma corticosterone in depression. Psychoneuroendocrinology 15, 485–488.

Shaarawy, M., Fayad, M., Nagui, A.R., Abdel-Azim, S., 1982. Serotonin metabolism and depression in oral contraceptive users. Contraception 26, 193–204.

Stout, S.C., Nemeroff, C.B., 1994. Stress and psychiatric disorders. Semin. Neurosci. 6, 271–280.

Tennant, C., 2002. Life events, stress and depression: a review of recent findings. Aust. N. Z. J. Psychiatry 36, 173–182.

Tilders, F.J.H., Berkenbosch, F., 1986. CRF and catecholamines; their place in central and peripheral regulation of distress response. Acta Endocrinol., Suppl. 276, 63–75.

Van der Hart, M.G., Czeh, B., de Biurrun, G., Michaelis, T., Watanabe, T., Natt, O., Frahm, J., Fuchs, E., 2002. Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cerebral metabolites, cytogenesis in the dentate gyrus and hippocampal volume. Mol. Psychiatry 7, 933–941.

Van Wimersma Greidanus, T.B., Bohus, B., De Wied, D., 1974. The parafasicular area as the site of action of ACTH analogs on avoidance behavior. Prog. Brain Res. 41, 429–432.

Van Wimersma Greidanus, T.B., Van, Bohus, B., De Wied, D., 1975. The role of vasopressin in memory processes. Progr. Brain Res. 42, 135–141.

Weiss, J.M., McEwen, B.S., Silva, M., Kalkut, M., 1970. Pituitary adrenal alterations and fear responding. Am. J. Physiol. 218, 864–868.

Willner, P., 1984. The validity of animal models of depression. Psychopharmacology 83, 1–16.

Wilson, M.A., Burghardt, P.R., Ford, K.A., Wilkinson, M.B., Primeaux, S. D., 2004. Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. Pharmacol. Biochem. Behav. 78, 445–458.

Zebrowska-Lupina, I., Stelmasiak, M., Porowska, A., 1990. Stress-induced depression of basal motility: effects of antidepressant drugs. Pol. J. Pharmacol. Pharm. 42, 97–104.