ORIGINAL ARTICLE

Effects of noradrenaline and serotonin reuptake inhibitors on pituitary-adrenocortical and sympatho-adrenomedullar system of adult rats

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Submitted: July 16, 2007 *Accepted: August 8, 2007*

Key words: **chronic mild unpredictable stress; catecholamines; CORT; ACTH; antidepressant**

Neuroendocrinol Lett 2007; **28**(5):614–620 **PMID:** 17984940 NEL280507A09

Abstract

OBJECTIVE: To estimate the influence of long-term treatment with noradrenergic and serotonergic reuptake inhibitors on activity of pituitary-adrenocortical and sympatho-adrenomedullar systems in animals, we compared the effects of maprotiline (a selective inhibitor of noradrenaline reuptake) and fluxilan (a selective inhibitor of serotonin reuptake) on plasma noradrenaline (NA), adrenaline (A), adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels in unstressed control and rats exposed to chronic unpredictable mild stress (CUMS).

METHODS: Plasma NA and A were assayed by a radioenzymatic method. Plasma CORT was measured using RIA kits and plasma ACTH by a chemiluminescent method.

RESULTS: CUMS did not affect blood plasma NA, A and ACTH content, but elevated plasma CORT level. Maprotiline elevated plasma NA content both in unstressed control and CUMS group, whereas plasma A remained unchanged. Fluxilan acted significantly increasing plasma NA and A concentrations both in control and CUMS rats. Neither maprotiline nor fluxilan affected plasma ACTH level both in unstressed control and CUMS animals. Plasma CORT level in unstressed control rats remained unchanged after maprotiline and fluxilan treatment, while being significantly decreased in CUMS rats.

CONCLUSION: Chronic treatment of adult rat males with maprotiline, a noradrenaline reuptake inhibitor activated sympathoneural system. On the other hand, fluxilan, a serotonin reuptake inhibitor activated both sympathoneural and adrenomedullar system, whereas both antidepressants desensitized HPA axis. The findings described here suggest that elevated plasma catecholamines my contribute to adverse effects of these drugs on cardiovascular parameters during antidepressant therapy.

Abbreviations

A	- adrenaline
ACTH	- adrenocorticotropic hormone
COTR	- corticosterone
CRH	- corticotropin-releasing hormone
CUMS	- chronic unpredictable mild stress
HPA	- hypothalamo-pituitary-adrenocortical axis
NA	- noradrenaline
POMC	- preproopiomelanocorticotropin

INTRODUCTION

Stress-induced activation of sympatho-adrenomedullar system results in adrenaline (A) and noradrenaline (NA) release followed by hypothalamo-pituitary-adrenocortical axis (HPA) activation and the release of adrenocorticotropic hormone (ACTH) and corticosterone (CORT) (Gavrilovic and Dronjak, 2005). Prolonged, chronic stress-induced elevation of catecholamine and CORT levels could be an important factor in stress-related pathology, including depression. Neuroendocrine system is a target for antidepressant drug action. Antidepressants affect monoaminergic neurotransmission and modulate central neuropeptides involved in the coordination of stress response and the control of HPA axis activity. While acute administration of antidepressants was shown to result in a rise of hormone secretion, chronic treatment expressed opposite effects. So, decreased plasma CORT, ACTH, NA and A levels were observed after chronic treatment with several antidepressants (Holsboer and Barden, 1996; Jongsma *et al*. 2005). In contrast, some authors described a stimulatory action of antidepressants on plasma CORT, A, NA and dopamine concentrations (Durand *et al*. 1999; Jezova *et al*. 2002; Blardi *et al*. 2005). Both serotonin and NA are involved in the mechanism of action of most antidepressant drugs by mediating antidepressant response. Noradrenergic and serotonergic antidepressants have been associated with somewhat different clinical effects. It has been believed that the former express prominent effects on the motivation and drive, while the latter have beneficial effects on anxiety and mood in depressed patients (Montgomery 1995,1997). Dazzi *et al*. (2002) showed that long-term treatment with different antidepressants, able to potentiate serotonergic and/or noradrenergic transmission, markedly reduced the sensitivity of cortical noradrenergic neurons to acute footshock stress. In contrast, Page and Abercrombie (1997) reported that chronic treatment with fluoxetine, a selective serotonin reuptake inhibitor, potentiates stress-induced NA output. Also, Dazzi *et al*. (2005) found that long-term but not acute fluvoxamine administration completely antagonized footshock stress-induced increase in extracellular concentration of cortical serotonin, while failed to modify the sensitivity of cortical noradrenergic neurons to the same stress. The neurochemical and behavioral effects of reduced central neurotransmitter function and subsequent influence of antidepressants are difficult to study in humans for ethical reasons. Because of that, induced chronic stress in animals has been used as a model of depression. Chronic exposure of rats to mild and unpredictable stressors (termed **C**hronic **U**npredictable **M**ild **S**tress – CUMS), shown to produce behavioral changes similar to human depression, has been accepted as a valid and useful experimental model of depression (Willner 1992,1997). Numerous experimental studies demonstrated a direct connection between exposure to stress and the disease. However, little is known about stress effects on antidepressant drug action, as well as on combined effects of stress and different serotonergic and noradrenergic antidepressants on the release of A, NA, ACTH and CORT.

To estimate the influence of long-term treatment with noradrenergic and serotonergic reuptake inhibitors on the activity of pituitary-adrenocortical and sympatho-adrenomedullar systems in animals, we compared the effects of maprotiline (a selective NA reuptake inhibitor) and fluxilan (a selective serotonin reuptake inhibitor) in rats exposed to CUMS on plasma catecholamines, ACTH and CORT.

MATERIALS AND METHODS

Animals

Adult Wistar rat males weighing 280–320 g at the onset of experiments and maintained in a temperature-controlled room (21±1.0 °C) and 12 h/12 h light/dark cycle, were used. They were habituated to handling and treated as humanly as possible, according to the recommendations of the Helsinki Declaration and Guide for Care and Use of Laboratory Animals of the National Institute of Health (Bethesda, MD, U.S.A).

Drugs and chronic treatment protocols

The rats were randomly divided into control (unstressed) and CUMS group. These two groups were further divided into three subgroups each, and the animals were receiving daily injections of: 1. vehicle (sterile water); 2. maprotiline (10 mg/kg) or 3. fluxilan (10 mg/kg) by i.p. route. Exposure to CUMS and the vehicle, i.e. drug administration started on the same day and were continued for 4 weeks. Maprotiline (Sigma-Aldrich Chemie, Germany) and fluxilan (Aeigis LTD, Cyprus) solutions in sterile water, sonicated for approximately 10 min were prepared *ex tempore*.

Chronic unpredictable mild stress (CUMS)

The CUMS procedure, a slight modification of the method by Grippo *et al*. (2002) was designed to maximize the unpredictable nature of the stressors. The CUMS groups were exposed to the following stressors in random order: continuous illumination (24 h), continuous darkness (24 h), 40 ° cage tilt along the vertical axis, crowding (8 rats *per* cage), soiled cage (300 ml water spilled onto the bedding), restraint in a small cage, cold room (4 °C), individual housing (24 h), forced running (15 min), food and water deprivation. Animals were also

Table 1. Chronic unpredictable mild stress (CUMS) procedure.

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	SATURDAY/ SUNDAY
WATER DEPRIVATION			8:00–15:00			REVERSED LIGHT / DARK CYCLE
FOOD DEPRIVATION			15:00 →	8:00		
45 ° CAGE TILT				15:00 →	8:00	
SOILED CAGE		15:00 →	8:00			
CROWDING (8 rats per cage)	8:00 →	8:00				
INDIVIDUAL HOUSING			8:00 →	8:00		
RESTRAINT CAGE				1 h		
COLD ROOM (4 °C)					9:00–14:00	
FORCED RUNNING	20 min–30 m/s					
24 h CONTINUOUS ILLUMINATION				8:00 →	8:00	
24 h CONTINUOUS DARKNESS		8:00 →	8:00			

maintained on a reversed light/dark cycle from Friday evening to Monday morning. The details on the CUMS procedure applied for a single week and repeated during the following 3 weeks, are listed in Table 1.

Biochemical analyses

Blood plasma catecholamines measured by a modified radioenzymatic assay after Peuler and Johnson (1977) were converted to their labelled O-methylated derivatives by S-(^{3}H)-adenosylmethionine (Perkin Elmer LAS, Inc., Boston, MA),and lyophilized catechol-O-methyltransferase isolated from rat liver. The resulting O-methylated derivatives were extracted along with unlabelled carrier compounds.

After prior extraction, plasma CORT levels were measured by RIA using commercial kits (MP Biomedicals, Eschwege, Germany).

Plasma ACTH content was determined by chemiluminescent method using an IMMULITE automatic analyzer (DPC, Los Angeles, CA, USA).

Statistics

Statistical analyses included Student's t-test and two-way ANOVA test. Data expressed as mean ± SEM represent an average of 6 animals. Statistical significance was accepted at $p<0.05$.

RESULTS

The effects of maprotiline and fluxilan on the blood plasma levels of NA of unstressed control and CUMS rats are presented in Figure 1. As seen, CUMS elevated plasma NA comparing to the control, but the difference was not statistically significant. Maprotiline acted elevating plasma NA 2-fold ($p<0.01$) and 1.5-fold in unstressed control and CUMS group ($p<0.05$), respectively, comparing to vehicle-treated control. Plasma concentration of this catecholamine after fluxilan administration was significantly increased (approx. 3.5-fold; $p<0.001$) in control and 2.3-fold in CUMS group ($p<0.01$) as compared to the corresponding vehicle-receiving controls. A two-way repeated measure analysis of variance (ANOVA, treatment × groups) showed that both treatments expressed a significant effect (maprotiline – $p<0.01$; fluxilan – $p<0.001$). In addition, the interaction between these two factors was also detected ($p<0.05$ and $p<0.01$, for maprotiline and fluxilan, respectively).

As shown in Figure 2, neither 28 days of CUMS nor maprotiline treatment affected the plasma level of A. However, A concentrations after fluxilan administration were increased in unstressed control and CUMS rats (about 2.8-fold, $p<0.001$ and 2.3-fold, $p<0.001$, respectively) in comparison with vehicle-receiving animals. A

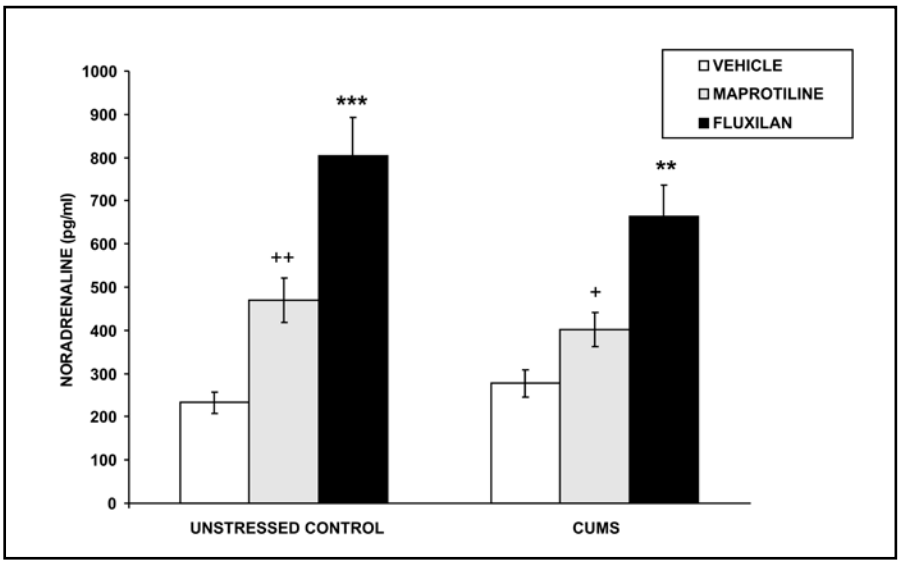

Figure1. Effects of long term administration of maprotiline and fluxilan on plasma levels of noradrenaline in unstressed adult rat males and those exposed to chronic unpredictable mild stress (CUMS).
The values are means ± SEM of 6 rats. Statistical significance: + p<0.05; ++p<0.01 maprotiline vs. vehicle; ** p<0.01; fluxilan vs. vehicle ***p<0.001.

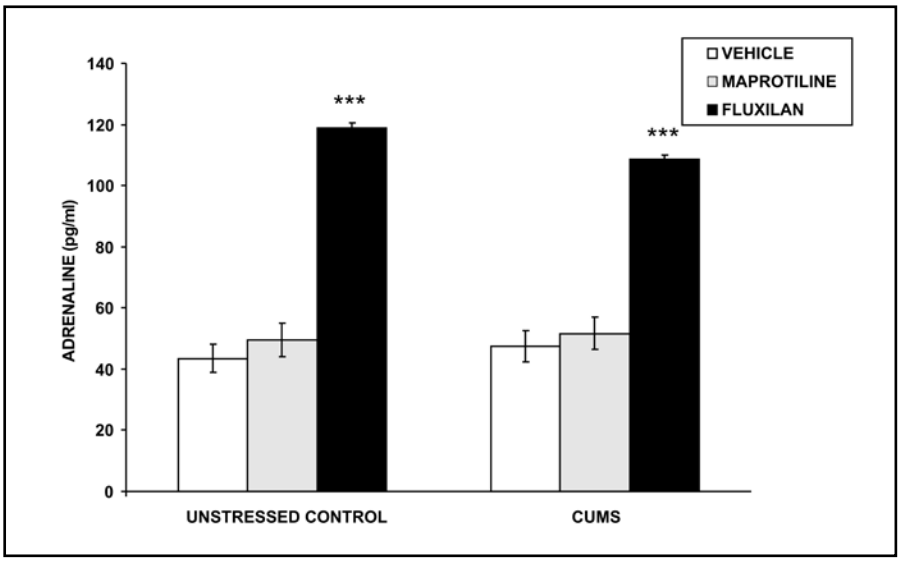

Figure 2. Changes in plasma adrenaline levels after long-term treatment of unstressed and CUMS rats with maprotiline and fluxilan.
The values are means ± SEM of 6 rats. Statistical significance: *** p<0.001 fluxilan vs. vehicle.

two-way repeated measure analysis of variance (ANOVA, treatment × groups) revealed a significant effect of fluxilan treatment (p<0.001).

CUMS did not affect plasma ACTH content. The same holds true for maprotiline- and fluxilan-treated unstressed control animals. However, both maprotiline and fluxilan acted decreasing plasma concentration of ACTH in CUMS rats (p<0.05) (Figure 3).

Changes in plasma CORT level of unstressed control and CUMS groups of rats are depicted in Figure 4. As seen, CUMS acted elevating plasma CORT content (p<0.05). On the other hand, maprotiline and fluxilan expressed no effect on plasma CORT concentration in control animals, but decreased CORT level was recorded in CUMS animals treated with either of these two antidepressants (p<0.05).

DISCUSSION

Noradrenergic and serotonergic systems have been implicated as important components of the central neurotransmitter network that plays a role in adaptation to stress (Stanford, 1996; Chaouloff, 2000). Antidepressant selectivity has traditionally focused on the neurotransmitters believed to be involved in depression. There are some discrepancies in the results of comparative studies related to selective effects of NA-selective (maprotiline, desipramine or amitriptyline) and serotonin-selective antidepressants (fluoxetine, paroxetine or citolopram) on brain monoaminergic neurons during stress (Shachar *et al*. 1997; Fujii *et al*. 2004).

In the present study, the effects of maprotiline, a NA reuptake inhibitor and those of fluxilan, a serotonin reuptake inhibitor on peripheral sympatho-adrenomedullar

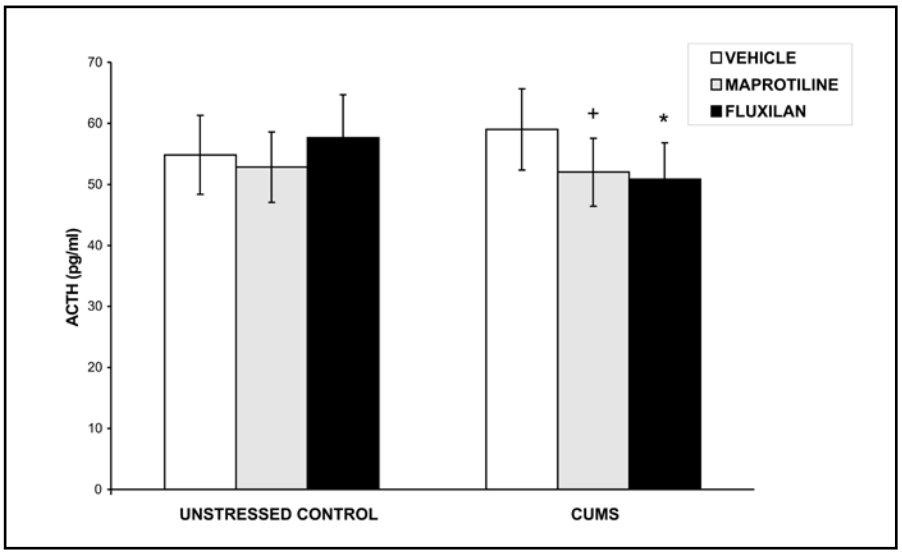

Figure 3. Influence of chronic treatment with maprotiline and fluxilan on plasma adrenocorticotropic hormone levels in unstressed and CUMS animals. The values are means ± SEM of 6 rats. Statistical significance: + p<0.05 maprotiline vs. vehicle; * p<0.05 fluxilan vs. vehicle.

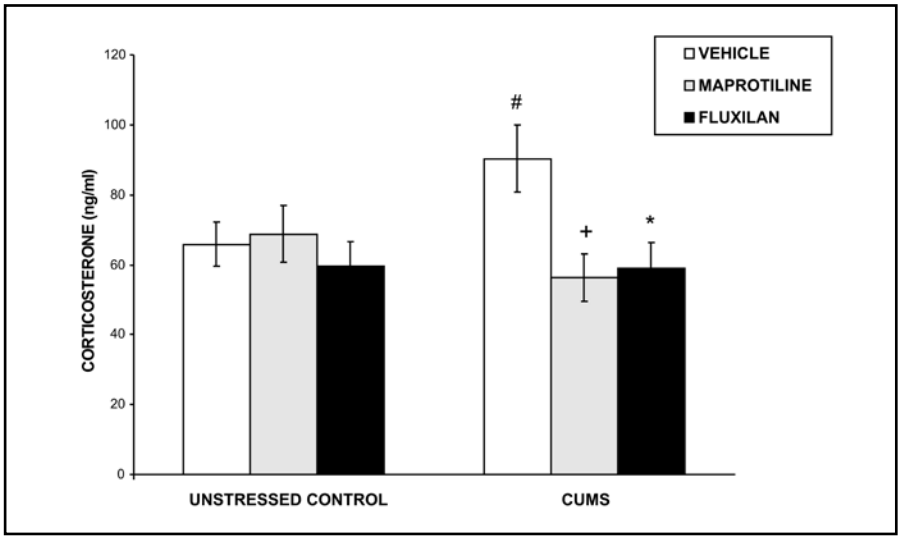

Figure 4. Changes in plasma corticosterone levels after chronic administration of maprotiline and fluxilan to unstressed and CUMS rats. The values are means ± SEM of 6 rats. Statistical significance: #p<0.05 unstressed vehicle-receiving control vehicle vs. CUMS group receiving vehicle; + p<0.05 maprotiline vs. vehicle; * p<0.05 fluxilan vs. vehicle.

and pituitary-adrenocortical systems were compared. For that purpose, plasma NA, A, ACTH and CORT levels in unstressed control and CUMS rats treated with maprotiline or fluxilan were determined. Our results showed that CUMS produced no significant increase of either NA or A plasma levels. Maprotiline treatment led to a gradual increase of plasma NA concentration in control and CUMS rats, but plasma A content remained unaffected. However, long term fluxilan treatment produced a pronounced increase in the content of both plasma NA and A both in unstressed control and CUMS rats. At the moment, it is rather difficult to explain why maprotiline acted inducing only an increase of plasma NA content. It is possible that the observed elevated plasma NA after maprotiline resulted from its release from sympathetic nerve endings. It is also a question which mechanism(s) of action is involved in fluxilan interaction with catecholamines leading to a pronounced enhancement of plasma content of both NA and A. Jensen *et al.* (1995) indicated that serotonin immunoreactive nerve fibres, *via* direct synaptic contacts, affect the activity of the vast majority of sympathetic preganglionic neurons that send axons either to the superior cervical ganglion or to the adrenal medulla. This serotonin input may be sympathoexcitatory and could mediate increase in sympathetic nerve activity and the release of catecholamines from the adrenal medulla. Alternatively, fluxilan-induced increase of plasma catecholamines observed in the present work could result from an indirect interaction of serotonin with catecholaminergic system. The finding that serotonin receptors, 5-HT_{1A} and 5-HT_3 in particular, can modulate the release of NA in rat brain (Mongeau *et al.* 1994; Gobert *et al.* 1997; Szabo *et al.* 1999) and that infusion of a

5-HT_{1A} receptor agonist increased plasma A (Korte *et al.* 1991), support this hypothesis. Circulating catecholamines secreted from the adrenal medulla belong to the group of main regulatory factors of car-

diovascular function. They are known to influence heart rate and peripheral vasoconstriction and are believed to play a role in pathophysiology of cardiovascular diseases. Fluxilan-induced elevation of plasma catecholamine level observed in rats treated with this serotonin uptake inhibitor can cause severe cardiovascular disturbances. Grippo *et al*. (2006) found that 4 weeks of fluoxetine treatment administered concurrently with 4 weeks of CUMS can prevent anhedonia but might only partially prevent increased cardiac sympathetic tone and attenuate heart rate.

In CUMS animals, the basal plasma CORT level was increased, whereas basal plasma ACTH content remained unchanged. These results could be connected to the data of Xia *et al*. (2006) who found that CUMS acted increasing serum corticotropin-releasing hormone (CRH) and cortisol concentrations, but did not change significantly serum ACTH level. The results of the present study demonstrated that long-term treatment with maprotiline did not affect plasma ACTH and CORT content in unstressed control rats. However, chronic maprotiline treatment of CUMS rats resulted in reduced plasma ACTH and especially CORT levels, suggesting a decreased activity of the HPA axis. This is in agreement with the reports of Inder *et al*. (2001) who observed that chronic treatment with antidepressants restored HPA axis hyperactivity in depressive patients and Reul *et al*. (1993) who found that this treatment reduced basal levels of CORT and ACTH. It is likely that maprotiline acted similarly to other NA reuptake inhibitors, *e.g.* desipramine. Desipramine was shown to reduce the activity of the *locus coeruleus*, a major source of NA innervation of the hypothalamic paraventricular nucleus, the site of CRH neurons. It also acted decreasing CRH mRNA expression and attenuating plasma ACTH and CORT (Brady *et al*. 1991; Torpy *et al*. 1995).

The results obtained throughout the present study showed that chronic maprotiline treatment expressed no effect on plasma ACTH and CORT levels in unstressed controls, but decreaseed their content in CUMS rats. The same holds true for fluxilan effects. These findings could be related to the data of Kelliher *et al*. (2003), who found that chronic administration of NA-selective reuptake inhibitor talsupram and Lu 10-134-C, a serotonin reuptake inhibitor, expressed no effect both on basal serum CORT concentration and anterior pituitary preproopiomelanocorticotropin (POMC) mRNA expression. Our results clearly demonstrated that long-term fluxilan administration led to attenuation of ACTH and CORT release in CUMS rats. This attenuation could be due to the modulation of glucocorticoid or mineralocorticoid receptors induced by prolonged fluxilan treatment. It has been suggested that antidepressants may facilitate glucocorticoid receptor activation which can lead to increased negative feedback to circulating glucocorticoids. In this respect, the enhancement of glucocorticoid receptor function, but not of the corresponding gene expression, seems to be of a special importance (Pariante and Miller, 2001).

In conclusion, the obtained results showed that chronic treatment with maprotiline, a noradrenaline reuptake inhibitor activated sympathoneural system, while fluxilan, a serotonin reuptake inhibitor activated both sympathoneural and adrenomedullar systems, whereas continuous treatment with either of these two antidepressants desensitized HPA axis. The findings described here suggest that elevated plasma catecholamines my contribute to adverse effects of these drugs on cardiovascular parameters during antidepressant therapy. Finally, the present study adds to to our understanding of the interactions between different antidepressants and stress-induces endocrine alternations.

ACKNOWLEDGEMENT

This work was supported by the Ministry for Science, Technology and Environmental Protection of Serbia, Contract No. 143044B

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