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ENDOCRINOLOGICAL AND BEHAVIOURAL EFFECTS OF CHRONIC FLUXILAN ADMINISTRATION IN RATS

DEJSTVO HRONIČNOG TRETMANA FLUKSILANOM NA PONAŠANJE I ENDOKRINE PARAMETRE KOD PACOVA

Nataša Spasojević[1], Ljubica Gavrilović[1], Ivan Kovačević[2], Slađana Dronjak[1]

[1]Institute of Nuclear Sciences »Vinča«, Laboratory of Molecular Biology and Endocrinology, Belgrade, Serbia
[2]Medicines and Medical Devices Agency of Serbia, Belgrade, Serbia

Summary: Chronic stress induces changes in the neuroendocrine and neuronal system, including elevation of catecholamines and corticosterone (CORT) levels, and could be an important factor in initial depression. Antidepressants affect monoaminergic neurotransmission and modulate central neuropeptides involved in the coordination of stress response and the control of HPA axis activity. We studied the effects of chronic treatment with fluxilan, a selective inhibitor of serotonin reuptake, in unstressed controls and chronic unpredictable mild stress (CUMS) rats, on behaviour and plasma noradrenaline (NA), adrenaline (A), corticosterone (CORT) and adrenocorticotropic hormone (ACTH). CUMS did not affect plasma NA, A and ACTH, but elevated plasma CORT content. Plasma concentration of catecholamines after fluxilan administration was significantly increased in control and CUMS group. On the other hand, fluxilan expressed no effect on plasma ACTH and CORT concentrations in control animals, but decreased ACTH and CORT levels in CUMS animals. Behaviourally, fluxilan treated animals displayed enhanced anxiety. The results demonstrate that the anxiogenic effects of chronic fluxilan administration are similar to those reported by many other studies. The findings described here suggest that elevated plasma catecholamines may contribute to an adverse effect of this drug on cardiovascular parameters during antidepressant therapy.

Key words: depression, catecholamines, corticosterone, adrenocorticotropic hormone, antidepressant

Kratak sadržaj: Hroničan stres indukuje promene u neuroendokrinom i nervnom sistemu, dovodeći do povećanja nivoa kateholamina i kortikosterona, i može biti važan faktor u nastanku depresije. Antidepresivi utiču na monoaminergičku neurotransmisiju i modulišu centralne neuropeptide uključene u koordinaciju odgovora na stres i kontrolu aktivnosti HPA ose. Ispitivano je dejstvo hroničnog tretmana fluksilanom, selektivnim inhibitorom ponovnog preuzimanja serotonina, kod nestresiranih kontrola i pacova izloženih hroničnom nepredvidivom blagom stresu (CUMS) na ponašanje i nivo noradrenalina (NA), adrenalina (A), kortikosterona (CORT) i adrenokortikotropnog hormona (ACTH) u plazmi. CUMS nije povećao nivo NA, A i ACTH u plazmi, ali je povećao nivo CORT. Koncentracija kateholamina u plazmi posle tretmana fluksilanom bila je značajno povećana kako kod nestresiranih kontrola tako i kod CUMS pacova. Međutim, fluksilan kod nestresiranih kontrola nije menjao koncentracije ACTH i CORT, ali je kod CUMS životinja smanjio njihovu koncentraciju. Životinje tretirane fluksilanom pokazale su povećanje anksioznosti. Rezultati pokazuju da je anksiozno dejstvo dobijeno hroničnim tretmanom fluksilanom slično rezultatima dobijenim u drugim studijama. Dobijeni podaci ukazuju da povećan nivo kateholamina može pogoršati neželjena dejstva ovog leka na kardiovaskularne parametre tokom terapije antidepresivima.

Ključne reči: depresija, kateholamini, kortikosteron, adrenokortikotropni hormon, antidepresivi

Address for correspondence:
Slađana Dronjak, Ph.D.
Institute of Nuclear Sciences »Vinča«
Laboratory of Molecular Biology and Endocrinology
P.O.B. 522-090
11000 Belgrade, Serbia
Tel./Fax: 00381112455561
e-mail: sladj@vin.bg.ac.yu

Introduction

Although short-term activation of neuroendocrine and central nervous transmitter systems is essential for vital function, deleterious effects of their constant hyperactivity can be considered as maladaptive. As demonstrated in animal studies, chronic stress in-

duces changes in the neuroendocrine and neuronal system including elevation of catecholamines and corticosterone (CORT) levels, and could be an important factor in initial depression (1, 2). Neuroendocrine system is a target for antidepressant drug action. In the recent years, selective reuptake inhibitors have represented a consistent advance in the pharmacotherapy of depression since alterations of serotonin neurotransmission are known to play a pivotal role in the pathophysiology of depression (3, 4). It has been recognized that selective reuptake inhibitors act by selectively inhibiting the cell reuptake of serotonin (5) and exert a weak inhibitory activity on noradrenaline (NA) and dopamine (6, 7). Several authors have reported that in rat brain acute administration of fluoxetine increases not only extracellular serotonin but also NA (8–11). However, Dazzi et al. (12) 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 behavioural 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 behavioural changes similar to human depression, has been accepted as a valid and useful experimental model of depression (13, 14). Antidepressants acting on serotonin have been thought to have beneficial effects on anxiety in depressed patients (15). This view was supported by the observation that they appear to be effective across a spectrum of anxiety disorders (16). However, two studies described that fluoxetine-treated animals displayed enhanced anxiety (17, 18).

The aim of the present study was to examine the effects of chronic treatment with fluxilan, a selective inhibitor of serotonin reuptake, in unstressed controls and CUMS rats on behaviour and plasma NA, adrenaline (A), CORT and adrenocorticotropic hormone (ACTH).

Materials and Methods

Animals

Adult Wistar rat males, weighing 280–320 g at the onset of experiments and kept in a temperature-controlled room (21±1.0 °C) and on a 12 h/12 h light/dark cycle, were used.

Drugs and chronic treatment protocols

The rats were randomly divided into control (unstressed) and CUMS group. These two groups were further divided into two subgroups each, and the animals were receiving daily injections of: 1. vehicle (sterile water); 2. fluxilan (10 mg/kg) by i.p. route. Exposure to CUMS and the vehicle, i.e. drug administration, started on the same day and was continued for 4 weeks. 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. (19), 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 kept on a reversed light/dark cycle from Friday evening to Monday morning.

Biochemical analyses

Blood plasma catecholamines measured by a modified radioenzymatic assay after Peuler and Johnson (20) 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 6–9 animals. Statistical significance was accepted at $p < 0.05$.

The elevated plus-maze procedure

The plus-maze consisted of two open arms, 50 × 10 cm (length × width), and two enclosed arms 50 × 10 × 50 cm (length × width × height), arranged in such a way that the two arms of each type were opposite to each other. The maze was elevated to a height of 50 cm. The rats were placed individually in the center of the maze facing a closed arm and allowed 5 min of free exploration. The behaviour

of each animal in the maze was analyzed, taking into account the standard measures recorded in each section of the maze (closed and open arms, central platform), comprising the percent of open arm entries (arm entry defined as all four paws into an arm), total arm entries, and the percent of time spent by the animals in the open arms of the maze.

Results

As seen from *Table I*, CUMS elevated plasma NA comparing to the control, but the difference was not statistically significant. Plasma concentration of this catecholamine after fluxilan administration was significantly increased about 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. CUMS did not affect plasma A and ACTH content. 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. Fluxilan treatment in unstressed controls did not change plasma levels of ACTH, but fluxilan acted by decreasing plasma concentration of ACTH in CUMS rats ($p<0.05$). CUMS acted by elevating plasma CORT content ($p<0.05$). On the other hand, fluxilan expressed no effect on plasma CORT concentration in control animals, but a decreased CORT level was recorded in CUMS animals ($p<0.05$).

The results presented in *Table II* show that CUMS rats treated with vehicle significantly decreased ($p<0.01$) total arm entries, the percentage of entries into open arms and time spent in open arms compared to vehicle treated unstressed control rats. Animals treated with fluxilan in both unstressed control and CUMS rats displayed an increase in the percentage of open arms entries and percentage of time spent in open arms, but it was not statistically significant.

Discussion

In this study we investigated plasma catecholamines, CORT and ACTH after chronic administration of fluxilan to unstressed control and CUMS rats. We found that CUMS produced no significant increase of either NA or A plasma levels. However, chronic fluxilan treatment produced a pronounced increase in the content of both plasma NA and A both in unstressed control and CUMS rats. It is 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. (21) 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 sympatho-excitatory 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 the 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 (22–24) and that infusion of a 5-HT_{1A} receptor agonist increased plasma A (25), supports this hypothesis. Circulating catecholamines secreted from

Table I Effects of long-term administration of fluxilan on plasma levels of noradrenaline (NA), adrenaline (A), adrenocorticotropic hormone (ACTH) and corticosterone (CORT) levels in unstressed adult rat males and those exposed to chronic unpredictable mild stress (CUMS).

HORMONES conc.	NA(ng/mL)	A (ng/mL)	ACTH (ng/mL)	CORT (pg/mL)
Unstressed control + vehicle	233.17±24.18	43.50±4.54	54.83±6.46	65.83±6.29
CUMS + vehicle	277.00±34.74	47.50±5.08	59.00±2.11	90.33±9.49$^{\#}$
Unstressed control + fluxilan	804.17±87.82***	119.00±1.50***	57.67±6.99	
CUMS + fluxilan	664.67±70.38**	108.50±1.38***	50.83±1.60*	58.83±7.45*

Statistical significance:
– Unstressed vehicle-receiving control *vs.* CUMS group receiving vehicle
• # $p< 0.05$
– Fluxilan *vs.* vehicle
• * $p< 0.05$
• ** $p< 0.01$
• *** $p< 0.001$

Table II Effect of chronic fluxilan treatment on total arm entries, the percentage of entries into open arms and percentage of time spent in open arms of the elevated plus-maze.

Plus-maze test variable	Total arm entries	Percent of open arm entries	Percent of open arm time
Unstressed control + vehicle	6.50±0.76	18.67±2.49	6.33±1.05
CUMS + vehicle	2.00±0.52##	5.50±1.18##	1.16±0.20##
Unstressed control + fluxilan	3.83±0.60	24.50±2.32	7.76±0.99
CUMS + fluxilan	4.33±0.49	7.67±0.88	2.80±0.66

Statistical significance:
– Unstressed vehicle-receiving control vs. CUMS group receiving vehicle
• ## $p<0.01$

the adrenal medulla belong to the group of main regulatory factors of cardiovascular function. They are known to influence heart rate and peripheral vasoconstriction and are believed to play a role in the 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. (26) 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.

The results obtained throughout the present study showed that chronic fluxilan treatment expressed no effect on plasma ACTH and CORT levels in unstressed controls, but decreased their content in CUMS rats. This is in agreement with the reports of Inder et al. (27), who observed that chronic treatment with antidepressants restored HPA axis hyperactivity in depressive patients, and Reul et al. (28), who found that this treatment reduced basal levels of CORT and ACTH. Our results clearly demonstrated that long-term fluxilan administration led to an 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 special importance (29).

The elevated plus-maze is one of the most widely used animal models in contemporary preclinical research on anxiety (30–32). This model is based on the innate fear rodents have of open and elevated spaces. Stressed animals spent less time in open arms and longer time in closed arms (33). Evidence derived from clinical studies suggests that antidepressant drugs can effectively treat anxiety disorders. Recent research has suggested that noradrenergic and serotoninergic reuptake inhibitors are effective in this regard (34, 35). However, preclinical investigations with serotoninergic reuptake inhibitors in animal models of anxiety disorders reveal highly variable effects of these drugs (18). Chronic treatment with fluxilan did not change the percentage of open arm entries and of time spent in the open arms, suggesting an anxiogenic profile. These results could be connected to the data of Shishkina and co-workers (17) who found that chronic fluoxetine treated animals displayed enhanced anxiety and decreased locomotor activity. In conclusion, our results demonstrate that the anxiogenic effect of chronic fluxilan is similar to that reported by many other studies.

The obtained results showed that chronic treatment with fluxilan, a serotonin reuptake inhibitor, activated both the sympathoneural and adrenomedullar systems. The findings described here suggest that elevated plasma catecholamines may contribute to adverse effects of these drugs on cardiovascular parameters during antidepressant therapy.

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