STRESS-INDUCED CHANGES OF NEUROPLASTIC PROTEINS AND MODULATION BY CHRONIC ANTIDEPRESSANT TREATMENT

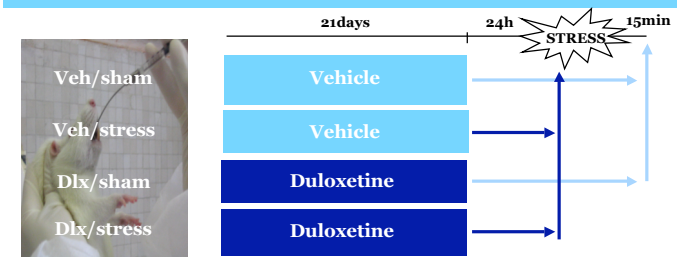
Calabrese F[1], Molteni R[1], Bolis F[1], Cattaneo A[2], Mancini M[3], Gennarelli M[2], Racagni G[1,2], Riva MA[1]

[1]Center of Neuropharmacology, Department of Pharmacological Sciences, University of Milan, via Balzaretti, 9, 20133 Milan, Italy;
[2]IRCCS Fatebenefratelli San Giovanni di Dio, Brescia;
[3]Eli Lilly italia S.p.A., Sesto Fiorentino, Italy.

INTRODUCTION

Although decreased levels of norepinephrine and serotonin may underlie depressive symptoms, compelling evidence now suggests that mood disorders are characterized by reduced neuronal plasticity. In fact, whereas antidepressant drugs rapidly enhance monoamine levels, their therapeutic effects are delayed by several weeks suggesting that adaptive changes may be required for therapeutic activity. Hence, pharmacological intervention may normalize such defects and improve neuronal function through the modulation of proteins and systems important for cellular plasticity and resiliency.
One important system in this context is the neurotrophin brain-derived neurotrophic factor (BDNF), whose expression and function is regulated by pharmacological treatments. However it is expected that effective antidepressants not only regulate basal expression of such proteins, but may modulate their responsiveness under stress, which represents an important factor of vulnerability in psychiatric conditions.
In the present study we investigated whether chronic treatment with the antidepressant duloxetine, a balanced serotonin-noradrenaline reuptake inhibitor, might alter the stress-induced modulation of BDNF (gene and protein expression) and molecules related to its signaling pathway in the rat hippocampus, a key region in mood disorders.

MATERIAL and METHODS



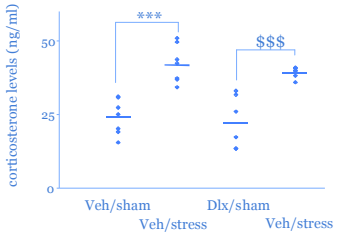
ANALYSIS OF RNA AND PROTEIN LEVELS
The hippocampus was dissected and used for the isolation of total RNA or protein. The analysis of BDNF mRNA levels were measured by real time PCR . Western blot analysis has been performed on total homogenate, in cytosolic, nuclear and in synaptosomal fractions.

PLASMA CORTICOSTERONE ASSAY
Samples of blood from each rat were collected in heparinized tubes. Plasma was separated by centrifugation (5000 rpm for 10 min) and corticosterone was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (IBL, Hamburg, Germany).

STATISTICAL ANALYSIS OF DATA
mRNA and protein levels have been calculated by measuring the signals' intensity of autoradiographs with the Quantity One software (Biorad). The values obtained were then normalized with respect to ß-actin used as internal standard.
Statistical analysis of the data were performed by two-way ANOVA with SCPHT. Data has been expressed as percentage versus Vehicle group (100%).

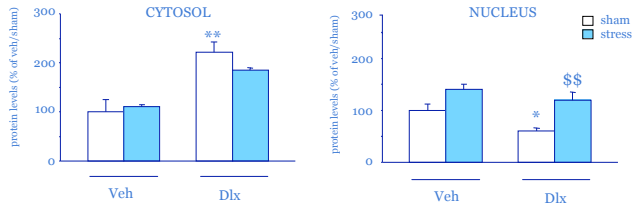
RESULTS

1. HORMONAL RESPONSE: corticosterone plasma levels



Corticosterone plasma levels measured in rats treated for 21 days with vehicle (Veh/Sham) or duloxetine (Dlx/sham), exposed to a forced swim 24 h after the last administration (Veh/stress and Dlx/stress) and killed 15 minutes after the end of the stress session.
Plasma corticosterone was increased by stress both in vehicle ($p<0.001$ with SCPHT) and in duloxetine-treated rats ($p<0.001$ with SCPHT) without any differences between the two experimental groups.
***$p<0.001$ vs. vehicle- and $^{\$\$\$}p<0.001$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

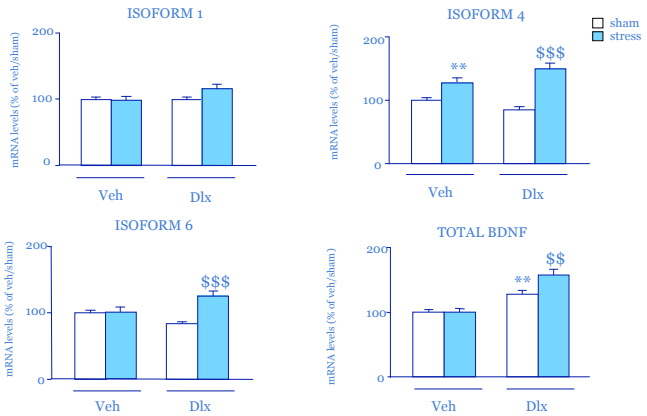
2. HORMONAL RESPONSE: glucocorticoid receptor levels



Effect of acute swim stress on glucocorticoid receptor protein levels in the cytosol and in the nuclear fraction obtained from the hippocampus of rats chronically treated with duloxetine (10 mg/kg) or vehicle and killed 15 minutes after the end of the stress session.
*$p<0.05$ and **$p<0.01$ vs. vehicle-treated rats; $$$p<0.01$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

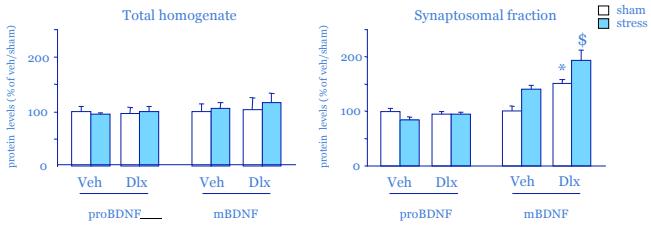
3. NEUROPLASTIC PROTEIN: BDNF

3a. gene expression: isoforms and total BDNF



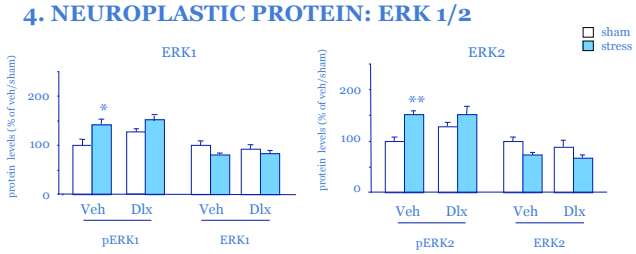
Total BDNF mRNA levels were significantly modulated by duloxetine as well as by stress, with a significant treatment X stress interaction. In fact, duloxetine treatment by itself up-regulated total BDNF mRNA levels. Exposure to swim stress increased the expression of BDNF coding exon only in duloxetine-treated rats but not in rats that were pre-treated with vehicle.
The analysis of specific BDNF splice variants indicates that while the levels of exon I transcript were not altered by antidepressant treatment or stress, the expression of exon IV- and exon VI-containing mRNAs was significantly modulated by our experimental paradigm. Specifically, with regard to BDNF exon IV mRNA levels we found a significant effect for duloxetine and stress but no drug X stress interaction. Even if duloxetine slightly reduced basal BDNF exon levels, stress up-regulated its mRNA levels in vehicle as well as in duloxetine-treated rats.
**$p<0.01$ vs. vehicle- and $^{\$\$}p<0.01$, $^{\$\$\$}p<0.001$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

3b. protein expression: BDNF in omogenate and synaptosomal fraction



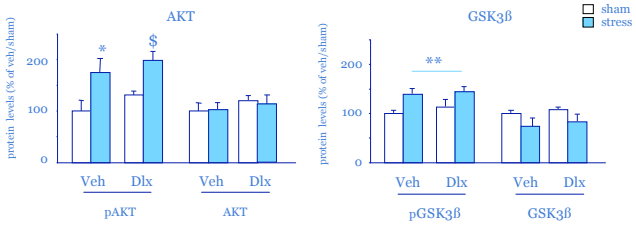
The hippocampal levels of proBDNF and mBDNF in whole homogenate were not affected by duloxetine treatment as well as by stress. However at synaptosomal level where mBDNF was significantly modulated by duloxetine and stress. Basal levels of mBDNF were significantly up-regulated by duloxetine treatment. Upon exposure to stress a significant increase of mBDNF protein levels was found in DLX-treated rats, but not in control animals.
*$p<0.05$ vs. vehicle- and $^{\$}p<0.05$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

4. NEUROPLASTIC PROTEIN: ERK 1/2



Effect of acute swim stress on ERK1 and ERK2 protein levels in the total omogenate obtained from the hippocampus of rats chronically treated with duloxetine (10 mg/kg) or vehicle and killed 15 minutes after the end of the stress session. Quantitative data represent the levels of the phosphorylated (P-ERK1 and P-ERK2, respectively) and the native (T-ERK1 and T-ERK2, respectively) forms expressed as a percentage of control values (un-stressed animals treated with vehicle, set at 100%).
*$p<0.05$ and * *$p<0.0001$ vs. vehicle-treated rats (two-way ANOVA with SCPHT).

5. NEUROPLASTIC PROTEIN: AKT and GSK3ß



Effect of acute swim stress on AKT and GSK3ß protein levels in the total omogenate obtained from the hippocampus of rats chronically treated with duloxetine (10 mg/kg) or vehicle and killed 15 minutes after the end of the stress session. Quantitative data represent the levels of the phosphorylated (P-AKT and P- GSK3ß, respectively) and the native (T-AKT and T- GSK3ß, respectively) forms expressed as a percentage of control values (un-stressed animals treated with vehicle, set at 100%).
*$p<0.05$ vs. vehicle-treated rats, $p<0.05$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

CONCLUSIONS

In summary, our results consolidate the idea that the neurotrophin BDNF may represent a common target of antidepressant treatment. Moreover, we provide evidence for a novel degree of modulation, which refers to the possibility that antidepressant drugs might enhance the synaptic pool of the neurotrophin and alter its signaling under challenging conditions, thus supporting the role of these pharmacological agents in the modulation of synaptic function and cellular resiliency.