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

Calabrese F¹, Molteni R², Bolis F³, Cattaneo A², Mancini M³, Gennarelli M³, Racagni G¹,², Riva MA³
¹Center of Neuropharmacology, Department of Pharmacological Sciences, University of Milan, via Balzaretti, 9, 20133 Milan, Italy;
²IRCCS Fatebenefratelli San Giovanni di Dio, Brescia;
³Eli Lilly Italy 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 is regulated 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-norepinephrine 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

PLASMA CORTICOSTERONE ASSAY

Samples of blood from each rat were collected in heparinized tubes. Plasma was separated by centrifugation (5000 rpm for 30 min) and in the supernatant corticosterone was determined by an enzyme-linked immunosorbent assay (ELISA) using a commercial kit (Biorad, USA) according to the manufacturer's recommendations.

RESULTS

1. HORMONAL RESPONSE: corticosterone plasma levels

Corticosterone plasma levels measured in rats treated for 2 days with vehicle (Veh/sham) or duloxetine (Dlx/sham), required to a basal stress or after the last administration (24 h after the last injection) and in rats injected after the end of the stress session. Plasma corticosterone was increased by stress levels in vehicle treated rats (two-way ANOVA with SCPHT; p<0.01 vs. basal stress and sham-stress rats). Treatment with duloxetine, respectively, normalized such stress-dependent changes (two-way ANOVA with SCPHT).

2. HORMONAL RESPONSE: glucocorticoid receptor levels

Effect of acute social stress on glucocorticoid receptor protein levels in the cytosol and in the nuclear fraction obtained from the hippocampus of rats chronically treated with vehicle (Veh/sham) or duloxetine (Dlx/sham) at the end of the stress session. p<0.01 vs. basal stress and sham-stress rats; d<0.05 in vehicle-treated rats (two-way ANOVA with SCPHT).

3. NEUROPLASTIC PROTEIN: BDNF

a. gene expression: isoforms and total BDNF

The hippocampal expression of proBDNF and mBDNF in whole homogenates were not affected by duloxetine treatment as well as by stress. However, proBDNF and mBDNF mRNA levels were significantly modulated by duloxetine and stress. Total levels of BDNF were significantly up-regulated by duloxetine treatment (two-way ANOVA with SCPHT; p<0.001 vs. basal stress and sham-stress rats). p<0.01 vs. vehicle- and $p<0.05$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

b. protein expression: BDNF in homogenate and synaptosomal fraction

The hippocampal levels of proBDNF and mBDNF in whole homogenates were not affected by duloxetine treatment as well as by stress. However, total BDNF protein levels were significantly modulated by duloxetine and stress. Total levels of BDNF were significantly up-regulated by duloxetine treatment (two-way ANOVA with SCPHT; p<0.001 vs. basal stress and sham-stress rats). p<0.01 vs. vehicle- and $p<0.05$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

4. NEUROPLASTIC PROTEIN: ERK 1/2

Effect of acute social stress on ERK1 and ERK2 protein levels in the total homogenate (two-way ANOVA with SCPHT; p<0.05 vs. sham-stress and basal stress rats). p<0.01 vs. vehicle- and $p<0.05$ vs. duloxetine-treated rats (two-way ANOVA with SCPHT).

5. NEUROPLASTIC PROTEIN: AKT and GSK3β

Effect of acute social stress on AKT and GSK3β protein levels in the total homogenate obtained from the hippocampus of rats chronically treated with vehicle (Veh/sham) or duloxetine (Dlx/sham) and killed 15 minutes after the end of the stress session. Quantitative data represent the levels of the phosphorylated (p-AKT and p-GSK3β, respectively) and the native (Akt and Y-GSK3β, respectively) forms expressed as a percentage of control values (un-stressed animals treated with vehicle, set at 100%). p<0.01 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.