VI Seminario Nazionale per Dottorandi in Farmacologia e Scienze Affini
Siena, Certosa di Pontignano, 23 - 26 Settembre 2002

EFFECTS OF IMMOBILIZATION STRESS ON EXPRESSION OF BRAIN-DERIVED NEUROTROPHIC FACTOR IN THE HIPPOCAMPUS OF TRANSGENIC MICE EXPRESSING TYPE II GLUCOCORTICOID RECEPTOR ANTISENSE RIBONUCLEIC ACID

Alboni S., 1° anno di corso del Dottorato in Scienze del Farmaco, a.a. 2001/2002 - III ciclo nuova serie. Durata del Dottorato in anni: 3. Sede d servizio: Dip.to Scienze Farmaceutiche dell'Università di Modena e Reggio Emilia via Campi n° 183, 41100 Modena.

Immobilization stress, a model of emotional stress, significantly enhances plasma corticosterone concentrations in experimental animals. The adrenal steroid corticosterone readily crosses the blood-brain barrier and thus directly affects the central nervous system (CNS). Glucocorticoids act by increasing or decreasing the transcription of specific target genes by binding to intracellular hormone receptors and forming a steroid-receptor complex which in turn can act as transcription factor. Two types of glucocorticoid receptors have been identified: the type I receptor (known as mineralocorticoid receptor - MR), and the type II receptor (or glucocorticoid receptor - GR). At basal hormone concentration, predominant MR occupation is observed, whereas increasing amounts of GR become occupied at the diurnal peak and after stress.
Of all brain structures that express GR and/or MR receptors, the hippocampus is particularly sensitive to adrenal steroids and stressful stimuli. In the hippocampus, a brain region implicated in learning, memory and mood disorders, the GRs e MRs are co-localized; furthermore glucocorticoids exert a wide range of effects on structure, function and survival of hippocampal neurons.
Neurotrophins have been indicated as important mediators of corticosterone action in the hippocampus, especially because these proteins appear to play a pivotal role in the development and maintenance of the nervous system. Stress, high concentrations or depletion of glucocorticoids influence the expression of neurotrophic and protective proteins and their receptors.

Since brain-derived neurotrophic factors (BDNF) is highly expressed in the hippocampus, the action of this neurotrophin is subject of intense study. For instance, many types of brain insults and stress result in modification in BDNF messenger ribonucleic acid (mRNA) expression in the rat hippocampus. In rats, single or repeated immobilization stress or exogenously applied corticosterone reduce BDNF mRNA and protein levels in the hippocampus. In contrast, exposure to single or chronic stress increases BDNF mRNA in hypothalamic nuclei. However, the effects of acute or chronic immobilization stress in mice are largely unknown.
The purpose of this study was therefore to investigate the effects of single rapid immobilization stress (30 minutes) on the expression of BDNF mRNA in the hippocampus of wild type (WT) and transgenic mice (TG) with impaired GR function. Impaired GR function was caused by endogenous expression of GR glucocorticoid receptor antisense RNA and insertion into the mouse genome of transgene expressing antisense RNA complementary to a fragment of the GR cDNA. These transgenic mice display impaired endogenous GR function and are characterized by dysfunctional glucocorticoid inhibitory feedback and disturbed ACTH and corticosterone secretory responses.
The effect of acute immobilization stress on BDNF mRNA expression in the hippocampus was assessed by RNAse protection assay.
Our results indicate that BDNF mRNA levels, in basal condition, do not significantly differ between WT mice and TG mice. However, rapid immobilization stress induces a significant increase of BDNF mRNA in the hippocampus of TG mice with respect to WT mice. Activation of the hippocampus and other brain regions by stress can be inferred by the activations of Immediately Early Genes such as c-fos, therefore c-fos mRNA can be used as a marker of neuronal activation. Taking into consideration that mice are in general less susceptible to stressful stimuli, c-fos induction was measured in the hippocampus in order to verify stress responsiveness. After 30 minutes of immobilization stress c-fos mRNA was significantly increased in WT as well as in TG mice, but the induction was significantly higher in the WT mice with respect to TG mice.
Those findings suggest that life-long central glucocorticoid receptor dysfunction results in an altered sensitivity with respect to immobilization stress. In the past, it has been difficult to ascribe GRs in the hippocampus to the effect of high plasma levels of glucocorticoids on BDNF mRNA expression. In these TG animals, characterized by partial knockout of GR, immobilization stress not only failed to inhibit BDNF mRNA expression, but induced the expression of BDNF mRNA.
The study of the mechanisms by which GC may regulate BDNF transcription is particularly important since BDNF downregulation may have consequences for the viability of hippocampal neurons. BDNF often exerts neuroprotective effects as opposed to glucocorticoids which in turn induce hippocampal cell death, thus the

mechanism underlying the effects of stress-induced alterations in BDNF mRNA may result in a functional protection of hippocampal neurons and even stimulate sprouting and synaptic reorganization both, necessary for adequate memory processes.
