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Original article

Effects of Different Psychotropic Agents on the Central Nerve Growth Factor Protein

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Abstract

Objective(s)
Psychotropic medications produce their effects, in part, through increasing neurotrophin levels in the brain. Since studies concerning nerve growth factor (NGF) analysis have been limited in scope, in the current experiments we investigated the effects of diverse psychotropic agents on NGF protein levels in various brain regions of rat.

Materials and Methods
Male Wistar rats received acute and chronic administration of drugs and electroconvulsive shock (ECS). Twenty four hr after the last treatment, NGF quantification was performed using sandwich ELISA kit.

Results
Acute administration of desipramine, phenelzine, fluoxetin, chlordiazepoxide (10 mg/kg, each), haloperidol (1 mg/kg), or clozapine (20 mg/kg) failed to alter NGF protein in any brain structure investigated. However, a single ECS treatment significantly elevated NGF protein in the hippocampus. Chronic administration (21 days) of desipramine, fluoxetine, phenelzine, haloperidol and clozapine led to a reliable enhancement of NGF protein in the frontal cortex. In addition desipramine, fluoxetine, phenelzine, and clozapine significantly increased NGF protein in the hippocampus. In the olfactory bulb, chronic injections of desipramine and fluoxetine elevated NGF level, however, phenelzine and haloperidol decreased NGF. Repeated applications of ECS (10 days) led to a remarkable augmentation of NGF protein in the frontal cortex, hippocampus, amygdala, and olfactory bulb. Neither acute nor chronic treatment with the benzodiazepine chlordiazepoxide altered NGF level in the examined brain regions.

Conclusion
These findings suggest that diverse psychotropic treatments may regulate NGF protein level in a brain region-specific fashion which may be indicative of their therapeutic properties.

Keywords: Brain, Electroconvulsive shock, Nerve growth factor, Psychotropic agents, Rat

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Introduction

The effects of psychotropic treatments on the norepinephrine (NE) and serotonin (5-HT) neurotransmitter systems in the brain are well known (1). Since the therapeutic actions of these agents are dependent on chronic treatment, increased levels of NE or 5-HT alone cannot account for their efficacy. Hence, alternative possibilities such as slow adaptive processes and intracellular signal transduction targets may be considered in this regard (2, 3). Over the past decade; increased neurogenesis, neuroplasticity and neuroprotection have become the focus of intense research especially in neuropsychiatric disorders associated with progressive brain tissue loss such as schizophrenia, bipolar disorder, and major depression (4, 5). In parallel, an interest in neurotrophins including NGF, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and their capacities to regulate central neurotransmission and promotion of neuroplasticity has been grown (6-8). There is ample evidence that neurotrophins exert numerous neuroprotective effects under pathological conditions which might be important in particular for neurodegenerative and psychiatric diseases (9). In a study conducted by Manev *et al*, antidepressant drugs improved information processing within neuronal networks through their neurotrophic effects (10). Accumulating preclinical and clinical data indicate that dysfunctions of NGF and BDNF may contribute to synaptic dysconnectivity and impaired brain development which lead to the schizophrenic syndrome or at least some of its presentations (11, 12). In rat brain associated with depression, atrophy was shown in several brain regions such as hippocampus, frontal cortex, and amygdala which was paralleled with the reduction of neurotrophic factor expression including NGF (13). It is also reported that NGF has profound effects on the growth, remodeling, synaptogenesis, and stability of dendrites and axons in hippocampal and cortical neurons (14). In general, the neurotrophic hypothesis of depression proposes that the etiology of depression and the action of antidepressant drugs are due, in part, to the regulation of central neurotrophin signaling. This has led to the investigation of the effects of different classes of antidepressants on the protein levels of neurotrophins in rat brain regions (15, 16). Vinay *et al*., have reported the different effects of typical and atypical antipsychotics on NGF expression in the cortex and nucleus basalis of rat (13). In addition, chronic ECS, an effective somatic treatment against drug-resistant depression, has been shown to increase NGF protein in the rhinal cortex and hippocampus (17-19). However, as compared to the regulation of BDNF levels which is most commonly studied, much less work has been done to analyze NGF protein level in different brain regions following administration of diverse psychotropic agents. Thus, the present study was designed to investigate the effects of acute and chronic treatments with different pharmacologic and somatic antidepressants on NGF protein levels in multiple brain regions of rat. To determine whether the regulation of NGF was unique to antidepressant treatments, antipsychotic and anxiolytic drugs were also considered.

Materials and Methods

Animals

Male Wistar rats weighing 250–300 g from our own animal facilities were used in this study. The animals were housed in pairs in polycarbonate cages and maintained on a 12 hr light/dark cycle in a temperature and humidity-controlled colony (22 °C). The animals were given free access to food pellets and water. The experimental procedures were conformed to the provisions of the Declaration of Helsinki and were also approved by the local Ethics Committee of AJA University of Medical Sciences.

Drug treatments

Dose and duration of drug treatment was selected based on the previous studies showing the elevation of BDNF or NGF mRNA (12, 13). Rats received once-daily subcutaneous injection of sterile saline (0.9%) or drug for either 1 day (n = 7 per group) or 21 days (n = 7). Drugs were dissolved in

sterile water and given in appropriate doses as follows: the tricyclic antidepressant (TCA); desipramine HCl (Sigma, St. Louis MO; 10 mg/kg), the selective serotonin reuptake inhibitor (SSRI); fluoxetine HCl (Anawa, Switzerland; 10 mg/kg), the monoamine oxidase inhibitor (MAOI); phenelzine sulfate (Sigma, St. Louis MO; 10 mg/kg), the benzodiazepine; chlordiazepoxide HCl (Sigma, St. Louis MO; 10 mg/kg), and the typical antipsychotic; haloperidol HCl (Tocris, Ellisville MO; 1 mg/kg). In addition, the atypical antipsychotic; clozapine (Tocris, Ellisville MO; 20 mg/kg) was dissolved in a small volume of acetic acid, adjusted to pH 5.2 with 10 N NaOH and brought up to final volume with sterile water. Control animals received only the vehicle (n=7).

Electroconvulsive shock

Rats were administered sham or ECS treatment either for 1 day (n = 7 per group) or 10 days (n = 7). To administer the ECS, the animal was lightly restrained by being wrapped in a paper towel with its head exposed. Conducting jelly was applied to the ears and electric current was administered between two earclip electrodes (50 mA, 0.5 Sec) using an electroshock generator (Ugo Basile, Italy). Sham-stimulated animals received the same treatment, except that no current was administered. This level and duration of shock produces a seizure that lasts less than 1 min and is characterized by full extension of the hind limbs (tonic phase) for 10-15 Sec, followed by repetitive flexion-extension of the forelimbs for 10-15 Sec (clonic phase). After the cessation of the shock, each rat was placed in a plastic cage where remained singly housed for 1 hr and returned to its home cage afterwards.

NGF quantification

Twenty-four hours after the last psychotropic treatment, rats were decapitated and their brains were quickly removed from the skull for dissection into the following regions: frontal cortex, hippocampus, amygdala, olfactory bulb, and brain stem. NGF protein levels were quantified using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Millipore, Billerica, MA). Briefly, each brain region was homogenized (1:10, w/v) in phosphate buffer solution (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycol bis(2-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA). Microtiter plates (96-well flat-bottom) were coated for 24 hr with the samples diluted 1:2 in sample diluent. Then, plates were washed four times with sample diluents. Monoclonal anti-NGF rabbit antibody diluted 1:1000 in sample diluent was incubated for 3 hr at room temperature. After washing, a second incubation with peroxidase-conjugated anti-rabbit antibody diluted 1:1000 was carried out for 1 hr at room temperature. After addition of streptavidin enzyme, substrate and stop solution, the amount of NGF was determined for absorbance in 450 nm. A standard curve, demonstrating a direct relationship between optical density and NGF concentration was considered which ranged from 7.8 to 500 pg of NGF. Total NGF protein was measured by Lowry's method using bovine serum albumin as a standard (20).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey’s test. Data are presented as mean±SEM. The level of significance was set at $P<0.05$.

Results

Effects of the psychotropic drugs on NGF protein levels

Acute administration of the drugs failed to change NGF protein levels in the examined brain regions (Figure 1, $P>0.05$). However, three weeks administration of desipramine, fluoxetine, phenelzine, haloperidol, and clozapine led to a significant enhancement of NGF protein in the frontal cortex (Figure 2, $P<0.05$, $P<0.05$, $P<0.001$, $P<0.05$, $P<0.01$, respectively). In addition; desipramine, fluoxetine, phenelzine, and clozapine increased NGF protein in the hippocampus (Figure 2, $P<0.01$, $P<0.05$, $P<0.05$, $P<0.001$,

respectively). In the olfactory bulb; desipramine and fluoxetine elevated NGF protein level (Figure 2, $P<0.05$, $P<0.01$, respectively), while, phenelzine and haloperidol caused a significant decrease of this neurotrophine (Figure 2, $P<0.05$, $P<0.05$, respectively). All drugs were unable to demonstrate significant change in the amygdala and brain stem as compared to the controls (Figure 2, $P>0.05$). Neither acute nor chronic treatment with the benzodiazepine chlordiazepoxide altered NGF levels in the selected brain regions (Figures 1 and 2, $P>0.05$).

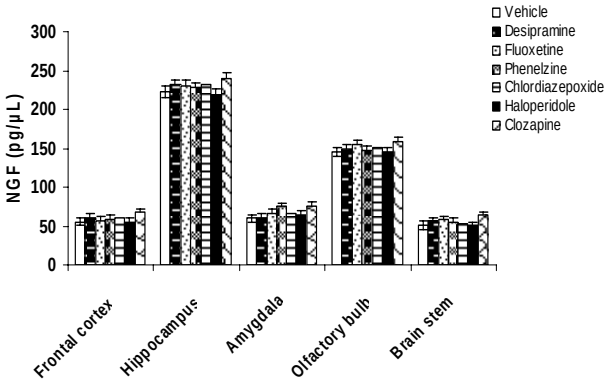

Figure 1. Effect of acute administration of psychotropic drugs on NGF protein level: Single injection of the drugs failed to alter NGF level in the brain regions investigated significantly, as compared to the control ($P>0.05$). Data are presented as mean±SEM.
Data obtained from clozapine vehicle group was similar to that of other drugs, thus, all were integrated.
pg/µl: picogram/microliter

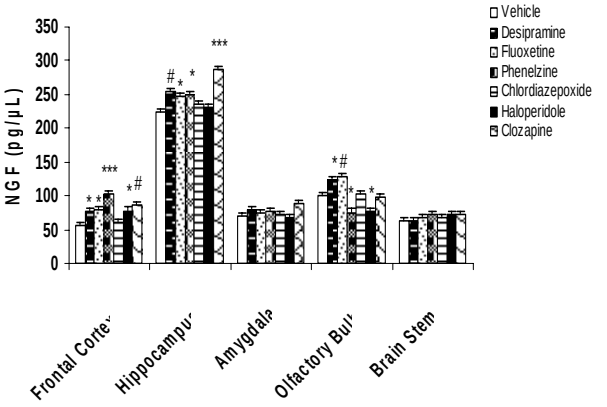

Figure 2. Effect of chronic administration of psychotropic drugs on NGF protein level: The drugs being able to regulate NGF protein level are shown. According to the ANOVA/Tukey test, all of the drugs were unable to demonstrate statistically significant change in the amygdala and brain stem as compared to the controls ($P>0.05$). Chronic treatment with the benzodiazepine chlordiazepoxide did not alter NGF levels in the selected brain regions. Data are presented as mean±SEM.
* $P<0.05$, # $P<0.01$, *** $P<0.001$ vs. control.

Effect of ECS on NGF protein levels

A single ECS treatment was sufficient to cause a significant increase of NGF protein in the hippocampus (Figure 3, $P<0.05$). Repeated applications of ECS resulted in a remarkable augmentation of NGF level in the frontal cortex, hippocampus, amygdala, and olfactory bulb (Figure 4, $P<0.001$, $P<0.001$, $P<0.01$, $P<0.01$, respectively).

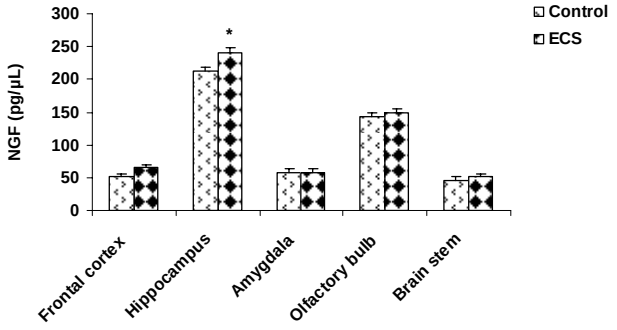

Figure 3. Effect of acute treatment with ECS on NGF protein level: A single ECS treatment led to a significant increase of NGF protein in the hippocampus, as compared to the sham-stimulated (control) animals. Data are presented as mean±SEM.

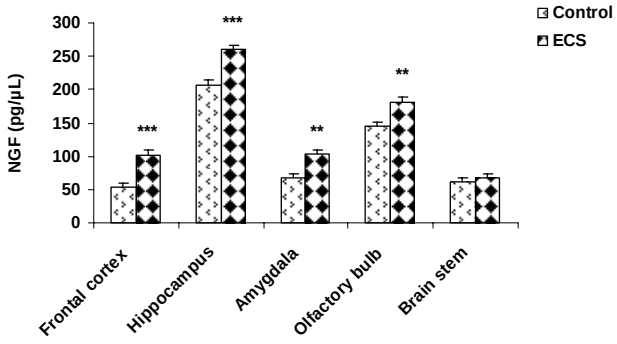

Figure 4. Effect of chronic treatment with ECS on NGF protein level: Ten days of ECS treatments, except for the brain stem, resulted to a significant augmentation of NGF in other brain structures examined, as compared to the sham-stimulated (control) animals. Data are presented as mean±SEM.
** $P< 0.01$, *** $P< 0.001$

Discussion

Over the recent years, after the previously dominating interest in the effects of psychotropic treatments on neurotransmitter levels in the brain, the focus of research has shifted towards the effects of these agents on intraneuronal signal transduction and cellular

resilience (2, 3). The discovery indicating that the psychotropic medications are neuroprotective and induce neurogenesis, has opened a new line of research in exploring the causes and treatments of neuropsychiatric diseases. Implication of neurotrophins in the mode of action of antidepressant drugs has been previously reported and is considered as an exciting new mechanism of action of these drugs (9, 12). Antidepressants have been shown to modulate growth factors even in cultured cells (10). In the present study, the effects of acute and chronic administration of different psychotropic agents on NGF protein levels in multiple brain regions of rat were investigated. According to the results, chronic treatment with pharmacologically distinct antidepressant drugs including desipramine, fluoxetine, and phenelzine significantly increased NGF protein levels in the frontal cortex (Figure 2). Frontal cortex is likely to be involved in depression and is sensitive to the effects of anti-stress and anti-depressant treatments (21). In postmortem studies of depressed patients, cellular and morphological changes including reductions in the number of glia and neuronal size of cortical structures have been observed (22). Similar changes have been reversed by chronic fluoxetine treatment in the cerebral cortex of rodents (23). As shown in Figure 2, chronic treatment with phenelzine induced elevation of NGF protein in the frontal cortex to a greater extent than either desipramine or fluoxetine. It is possible that phenelzine which inhibits the metabolism of all monoamines, might have greater effects on NGF protein levels than drugs which are more selective for specific monoamines. Previously, chronic administration of tranylcypramine; another MAOI, has been shown to increase NGF protein level in the frontal cortex (24). In the hippocampus, the aforementioned antidepressant drugs increased NGF (Figure 2). It is also known that, NGF regulates adult hippocampal neurogenesis (4). It is reported that hippocampal neurogenesis is one of those plastic processes that are regulated bi-directionally in response to either stress or antidepressant treatment (25-27). Meanwhile, phenelzine, in contrast to desipramine or fluoxetine, significantly reduced NGF level in the olfactory bulb (Figure 2). It appears that the regulation of NGF signaling, either positive or negative, could be involved in causing adaptive changes in neural plasticity induced by antidepressants.

As shown in Figures 1 and 2, neither acute nor chronic treatment with the anxiolytic chlordiazepoxide altered NGF level in any brain region investigated. Although anxiety can be effectively treated with SSRI antidepressants such as fluoxetine, the converse usually does not hold true. According to clinical data, patients with depression are generally not treated effectively with most benzodiazepine anxiolytics. Our data suggest that the inability of benzodiazepines to treat depression may, in part, be due to their inability to regulate central NGF levels.

Three weeks administration of the antipsychotics (haloperidol or clozapine), elevated NGF protein level in the frontal cortex (Figure 2). This, according to the sensitivity of the frontal cortex to the effects of antidepressant treatments (21), may be related to the efficacy of haloperidol or clozapine in treating depression. It is worth mention that antipsychotics have been recommended for the treatment of psychosis associated with depression (15). Meanwhile, the ability of antipsychotics to regulate central NGF levels has not been extensively investigated. Over the years, antidepressant treatments have been combined with antipsychotic drugs to treat bipolar and treatment-resistant depressions (28, 29). This may be due to the potential synergistic effects of both drug regimens on NGF protein levels. As shown in Figure 2, repeated injections of clozapine led to a remarkable augmentation of NGF protein in the hippocampus. It is reported that hippocampal dysfunction and/or atrophy are implicated in major neuropsychiatric disorders such as schizophrenia and mood disorders (4); therefore, clozapine via enhancement of the hippocampal NGF may increase neural proliferation and, perhaps, promote subsequent survival of neurons in the hippocampus, leading to the improvement of hippocampal function. This latter finding may justify the hippocampal neurogenesis or neuroprotection

induced by atypical antipsychotics (16, 28). However, the extent to which atypical antipsychotics including clozapine can modulate cell division or survival of newly generated cells within the hippocampus remains to be established. In a study conducted by Halim *et al*, administration of clozapine, 10 mg/kg for 28 days elevated NGF mRNA in sub-regions of the hippocampus (30). It is also well known that

NGF enhances cholinergic functioning and plays an important role in cognitive function (9). Meanwhile, the lack of effect of chronic treatment with antipsychotics on BNDF or NGF levels in the hippocampus has been reported by Valvassori *et al* (31). The discrepancy between our findings and those of Valvassori *et al* could be due to the differences in the duration of treatment, route of drug administration, and dosing regimen.

As shown in Figure 2, haloperidol had no appreciable effect on NGF protein levels in the hippocampus, as compared to clozapine. Thus, it seems that hippocampal neurogenesis is not affected by this typical antipsychotic. Several studies have indicated that haloperidol not only fails to stimulate neurogenesis in rats, but also appears to be neurotoxic by inducing apoptotic cell death that may be in part due to the decline of neurotrophins (32). In addition, chronic treatment with haloperidol decreased NGF protein level in the olfactory bulb (Figure 2). As previously shown, haloperidol produces mixed effects on NGF mRNA levels; either reduction in various hippocampal subfields with 1 mg/kg given for 28 days, or no change after 2 mg/kg given for 21 days (13, 16). At protein level, administration of haloperidol for 29 days reduced NGF in the hippocampus and frontal cortex (33). In general, it appears that second-generation antipsychotics in comparison with the first-generation drugs induce less deleterious effects on the levels of neurotrophic factors and cognitive function.

ECS, an effective somatic treatment against drug-resistant depression, was sufficient to cause a significant elevation of NGF protein level in the hippocampus following single administration (Figure 3), while, acute administration of pharmacological antidepressants did not alter NGF protein level in any brain structure investigated (Figure 1). Chronic application of ECS caused a more widespread up-regulation of NGF levels than the pharmacologic treatments (Figure 4). It appears that seizures evoked by ECS increase trophic factor expression which may serve as a neuroprotective function. This may be in parallel with the previous reports indicating that somatic antidepressant treatments including ECS, increase hippocampal neurogenesis and protect against apoptotic neuronal cell death via sustained enhancement of fibroblast growth factor-2 (FGF-2) and NGF mRNA (18). Over the years, various theories concerning the mechanism of action of ECS have been proposed including psychological, neurophysiological, neurochemical, neuroendocrine, and neuropeptide mechanisms. However, there is no single theory that satisfactorily explains the mechanism of ECS in various psychiatric illnesses. According to our data, it seems that the efficiency of ECS in drug-resistant depression might, in part, be linked to its regulatory effect on NGF protein levels in the brain.

Conclusion

The present work provide evidence that diverse pharmacologic and somatic antidepressant treatments, as well as antipsychotics, increase NGF protein level in a brain-region specific manner, even though they have different mechanisms of action at neurotransmitter systems.

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