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ORIGINAL RESEARCH

Decreased mRNA and Protein Expression of BDNF, NGF, and their Receptors in the Hippocampus from Suicide: An Analysis in Human Postmortem Brain

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Abstract: Despite the devastating effect of suicide on numerous lives, there is still a lack of knowledge concerning its neurochemical aspects. There is increasing evidence that brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are involved in the pathophysiology and treatment of depression through binding and activating their cognate receptors TrkB and TrkA respectively. The present study was performed to examine whether the expression profiles of BDNF and/or TrkB as well as NGF and/or TrkA were altered in the hippocampus of postmortem brain of the participants, who had committed suicide and whether these alterations were associated with specific psychopathologic conditions. These studies were performed on the hippocampus of 21 suicide victims and 19 non-psychiatric control individuals. The protein and mRNA levels of BDNF, TrkB, NGF, and TrkA were determined by sandwich enzyme-linked immunosorbent assay, Western blot and reverse transcription-PCR. Given the importance of BDNF and NGF and their cognate receptors in mediating physiological functions, including cell survival and synaptic plasticity, our findings of reduced expression of BDNF, TrkB, NGF, and TrkA on both the protein and mRNA levels of postmortem brains of suicide victims suggest that these molecules may play an important role in the pathophysiological aspects of suicidal behavior.

Keywords: brain-derived neurotrophic factor, nerve growth factor, suicide, postmortem brain, hippocampus, TrkB, TrkA, RT-PCR, ELISA

Introduction

Suicide is a major public health concern; however, its neurobiology is unclear. Postmortem brain tissue obtained from suicide victims irrespective of their status of depression and normal controls offers a useful method for studying the neurobiology of suicide. These studies, though preliminary, have offered important insights into the altered neurochemical milieu of suicide. In this article, we focus on the role of neurotrophins in suicide. Similarly to all other regions worldwide, suicide attempts in India have been increasing progressively. Despite the devastating effect of suicide on numerous lives, there is still a lack of knowledge concerning its underlying causes and pathologic mechanisms. Several clinical and epidemiological studies have identified stress as an important risk factor in suicide.[1,2]

The role of neurotrophins in directing brain growth and neuronal functioning is being increasingly recognized. Neurotrophins not only play an important role in cellular proliferation, migration, and phenotypic differentiation and/or maintenance in the developing central nervous system,[2,3] but also their presence is required in the adult central nervous system for maintenance of neuronal functions, structural integrity of neurons, and neurogenesis,[2,3] suggesting that neurotrophins are biologically significant over the entire lifespan. In addition, a number of studies have demonstrated that neurotrophic factors regulate structural and synaptic and morphological plasticity to modulate the strength or number of synaptic connections and neurotransmission.[4,5]

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays a key role in the development and survival of neurons in the central nervous system.[6] BDNF specifically binds to the tyrosine kinase receptor tropomyosin-related kinase B receptor (TrkB) and regulates many functions related to neuronal development such as neurite outgrowth, synthesis of differentiating factors and morphological plasticity.[6] In adulthood, BDNF is involved in neural homeostasis and in processes related to neuronal plasticity and connectivity, including learning and memory,[7,8] drug addiction,[9] response to social stress, aggressiveness, and anxiety-related behaviors.[10,11] Alterations in BDNF expression in specific neurons may reduce neural plasticity, therefore impairing the ability to respond to stressors and contributing to various neurodegenerative and neuropsychiatric disorders including depression and bipolar disorders.[12]

Nerve growth factor (NGF), the prototypical growth factor, is a protein secreted by a neuron's target cell. NGF is critical for the survival and maintenance of sympathetic and sensory neurons. NGF is released from target cells, binds to and activates its high-affinity receptor TrkA on the neuron, and is internalized into the responsive neuron. It also functions as a signaling molecule.[13,14] The NGF/TrkA complex is subsequently trafficked back to the neuron's cell body. This movement of NGF from axon tip to soma is thought to be involved in the long-distance signaling of neurons. NGF appears to play a role in the regulation of the hypothalamic-pituitary-adrenal (HPA) axis-mediated stress response, which exceeds its well-known neurotrophic function within the nervous system.

Results of many preclinical and clinical studies demonstrate that several types of stressors regulate the expression of BDNF and NGF in the brain.[15–22] In addition, preclinical and clinical study results indicate that they both may be involved in depression and in the mechanism of action of antidepressants.[23,28] Given the importance of BDNF and NGF in maintenance of the structural integrity and synaptic plasticity of the brain and their involvement in stress and affective disorders, we investigated the correlation of these neurotrophins with suicidal behavior by examining the expression of BDNF and NGF along with their cognate receptors in the postmortem brain of participants who committed suicide (Suicide participants) and nonpsychiatric healthy control individuals. Since BDNF and NGF mediate biological action through the activation of BDNF/TrkB and NGF/TrkA signaling cascades,[29–31] we examined alterations in the expression profiles of BDNF and/or TrkB along with NGF and/or TrkA in all suicide participants, irrespective of psychiatric diagnosis compared to healthy control participants.

In the present study, neurotrophins and their cognate receptors at both the protein and mRNA levels were compared in hippocampus obtained from suicide victims and non-psychiatric healthy control individuals.

Methods

Subjects

Postmortem brain samples from suicide participants who died by suicide with and without major

depression and non-psychiatric healthy control participants were obtained from the Calcutta Medical College Hospital under the supervision of the Institutional Ethical Committee. The hippocampal region of the brains were dissected and stored at −80 °C. We determined the selected neurotrophins and their respective receptors mRNA and protein expression in the hippocampal areas obtained from 19 non-psychiatric healthy control individuals and 21 suicide victims. Detailed demographic characteristics of control and suicide participants are shown in Table 1. The brain samples were free of any neuropathological abnormalities and human immunodeficiency virus. This study was approved by the Institutional Review Board (Ref. No. 06/B/IEC/MCH) of the Calcutta Medical College Hospital under West Bengal University of Health Sciences, Kolkata, India.

Diagnostic methodology

Psychiatric diagnoses of subjects were made using the psychological autopsy method. This technique has been validated for Axis I and II diagnoses.[32,33] Informants included primarily family member who were most familiar with the deceased such as the mother, father, siblings, friends, or relatives to serve as an informer and undergo the interview process. After giving written informed consent, at least one family member was interviewed using the Diagnostic Evaluation After Death (DEAD)[34,35] and the Schedule for Clinical Interviews for the DSM-IV (SCID)[36] by a psychiatrist. Information collected through SCID I and II interviews and from the coroner's notes and medical records were used by interviewers to write a case history for each subject. These case histories were then reviewed by a clinical panel in order to reach a consensus regarding the DSM-IV diagnosis for each subject. Similarly, controls were verified as free from mental illness using similar consensus diagnostic procedures.

The standard deviations (SD) of the mean age of non-psychiatric healthy control and suicide victims were 15.93 and 14.94. The age range was 15 to 80 years for normal participants and 15 to 75 years for suicide victims.

The study was performed on postmortem samples of brain tissue extracted from the hippocampal area obtained from 21 participants who died by suicide and 19 non-psychiatric healthy control participants.

Autoptic samples were collected within 12 to 26 h of the subject's death (postmortem interval information is reported in the Table 1), in the course of autopsy at the Calcutta Medical College Hospital Morgue, and stored at −80 °C. Data on subject's age range, drug history, and cause of death were gathered from the medical records of suicide victims. A detailed description of the subjects analyzed in this study is reported in the Table 1.

Extraction of hippocampus from suicide victims and non-psychiatric healthy controls

The brains of suicide participants and control individuals were removed for isolation of the hippocampal tissues. The hippocampus was cut into 0.5-cm coronal slices of the middle of the hippocampus, which included the dentate gyrus and areas CA1-4.

A total of 50–100 mg tissue of each subject from hippocampus was extracted using RIPA buffer [20 mM Tris-HCL (pH 8), 150 mM NaCl, 1 mM EDTA, 50 mM NaF, 1 mM Na_2MoO_4, 0.5 mM Na_3VO_4, 5 mM $Na_2P_2O_7$, 1% Triton X-100, 0.5% Na deoxycholate, 0.1% SDS, 10% glycerol, 10 μg/mL leupeptin, 10 μg/mL aprotinin, 0.01 mM phenylmethylsulfonyl fluoride, 1 mg/mL pepstatin A, and 10 mM benzamidine]. Supernatant was prepared by centrifugation (REMI, Mumbai, India) at $23792 \times g$ for 10 min at 4 °C. Protein content was determined using the Bradford method (Bio-Rad, Hercules, CA, USA).

Quantitative estimation of BDNF and NGF levels in the hippocampus of suicide victims and non-psychiatric healthy controls by sandwich ELISA

Endogenous BDNF and NGF levels were measured in hippocampus samples using an enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions (Chemicon, Temecula, CA, USA). Hippocampi were immediately extracted the method described above. Rabbit polyclonal antibodies generated against human BDNF and NGF were coated onto two separate microplates, which were used to capture BDNF and NGF, respectively, from the samples. BDNF-specific biotin-conjugated, mouse monoclonal antibodies were used to detect the captured BDNF and NGF separately. After addition

Table 1. Demographic characteristics of suicide victims and control individuals.

Subject no.	PMI (h)	Brain pH	Cause of death	Psychiatric diagnosis
Group: Participants died by suicide				
1	17.9	6.91	Acid poisoning	Familial disharmony (a type of psychosocial stressor)
2	24.8	6.23	Hanging	No psychiatric illness
3	13.9	6.72	Wrist cutting	Major depression, alcohol abuse
4	21.6	6.92	CuSO4 poisoning	Major depression, adjustment disorder
5	15.6	6.95	Hanging	No psychiatric illness
6	23	6.1	Jumped from multi storied building	Major depression
7	26.3	6.4	Hanging	Drug and alcohol abuse
8	18.8	5.69	Acid poisoning	Major depression
9	24.3	6.44	Hanging	Major depression, adjustment disorder
10	24	6.3	Wrist cutting	No psychiatric illness
11	24.8	6.65	Acid poisoning	Familial disharmony (a type of psychosocial stressor)
12	26.1	6.77	Multiple injuries	Drug and alcohol abuse
13	22	6.55	Jumped from multi storied building	Marital disharmony (a type of psychosocial stressor)
14	15.5	6.32	Hanging	Marital disharmony (a type of psychosocial stressor)
15	27	6.2	Hanging	No psychiatric illness
16	20.1	6.52	Jumped from multi storied building	Major depression, agoraphobia
17	11.5	7	Jumped in front of the metro rail	Familial disharmony (a type of psychosocial stressor)
18	24.7	6.66	Hanging	Bipolar disorder
19	19.3	7.06	Multiple injuries	Schizoaffective disorder
20	24.8	6.71	Wrist cutting	Major depression, adjustment disorder
21	24.5	6.25	Jumped in front of the metro rail	Familial disharmony (a type of psychosocial stressor)
Group: Control individuals				
22	22	5.8	Atherosclerotic cardiovascular disease	–
23	19.24	7.33	Accidental trauma	–
24	18.34	6.23	Cadiac arrhythmia	–
25	26.13	5.69	Hypertensive heart	–
26	27.23	5.89	Liver cirrhosis	–
27	15.5	6.35	Hypoplastic coronary artery	–
28	23	6.64	Cadiac arrhythmia	–
29	26.3	6.22	Atherosclerotic cardiovascular disease	–
30	18.8	5.98	Atherosclerotic cardiovascular disease	–
31	24.3	6.43	Pneumonia	–
32	29.13	5.33	Subarachnoid haemorrhage	–
33	18	7.17	Anaphylaxis	–
34	9.5	6.11	Mitral valve prolapse	–
35	17	5.44	Hypertensive heart	–
36	16	6.7	Hypoplastic coronary artery	–
37	10.32	6	Accidental trauma	–
38	17.5	6.11	Hypertrophic cardiomyopathy	–
39	21	5.7	Accidental trauma	–
40	24	6.47	Ovarian cancer	–

of streptavidin-enzyme substrate and stop solution, the amount of BDNF and NGF were determined. The standard curve showed a direct relationship between optical density (OD) and BDNF and NGF concentrations: i.e., a higher OD indicated higher the BDNF and NGF concentrations in the samples.

The amount of BDNF and NGF were determined by measuring the absorbance at 450 nm (Tecan Infinite M200, Mäennedorf, Switzerland). A standard curve was plotted, which ranged from 7.8–500 pg/mL of BDNF and NGF. These curves were obtained based on the direct relationship between optical density (OD) and neurotrophin concentrations. Total protein concentration was measured by Bradford method using bovine serum albumin (BSA) as a standard.

Immunoprecipitation of BDNF and NGF

Supernatant containing 100 μg of protein was incubated with antibodies against BDNF and NGF (100:1 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 2 h at 4 °C. A suspension of protein-A sepharose beads (Amersham, NJ, USA) in Tris-buffered saline (TBS) were added and incubated at 4 °C for 1 h. The pellet was collected by centrifugation at 2,500 rpm for 30 s at 4 °C and washed four times with TBS containing 0.5 mM Na_3VO_4 and 0.01 mM phenylmethylsulfonyl fluoride. The pellet was resuspended in 2× sample buffer, boiled for 5 min, and subjected to Western blot analysis.

Western blotting of hippocampal BDNF, TrkB, NGF, and TrkA proteins of suicide victims and non-psychiatric healthy controls

BDNF, TrkB, NGF, and TrkA were immunolabeled for Western blotting as described below. Equal volumes of soluble fractions containing 60 μg of proteins were electrophoresed on a 7.5% polyacrylamide gel and the proteins were transferred onto nitrocellulose membranes (Mini-PROTEAN® Tetra Cell with Mini-Trans Blot®, Bio-Rad). The membranes were blocked with 5% non-fat dried milk. Next, the membranes were incubated overnight at 4 °C with primary anti-BDNF polyclonal antibodies (Chemicon), (1:1000 dilution in 3% BSA) anti-TrkB polyclonal antibodies (Chemicon), (1:500 dilution in 3% BSA), NGF polyclonal antibodies (Chemicon), (1:1000 dilution in 3% BSA), or anti-TrkA polyclonal antibodies (1:400 dilution in

3% BSA, Santa Cruz Biotechnology). Nitrocellulose membranes were washed three times in 0.1% Tween-20 for 15 min each and incubated with horseradish peroxidase (HRP)-conjugated anti-sheep IgG (1:1000) for 2 h at room temperature. Immuno-reactive bands were visualized using enhanced chemiluminescence (ECL) (Santa Cruz Biotechnology). Membranes were stripped with stripping solution and probed with anti-β-actin monoclonal antibody (1:10,000 dilution in 3% BSA, Sigma, St. Louis, MO, USA), which was used as an internal control.

The OD value of each band was analyzed with the electrophoresis image analysis system (SmartView Pro Imager System, USA). The OD of each protein was determined by using the OD of the corresponding β-actin band. The values are presented as a percent of the control.

Isolation of total mRNA from the hippocampus of suicide victims and non-psychiatric healthy controls and RT-PCR of BDNF, NGF, TrkB, and TrkA

Hippocampus tissues were isolated from all subjects. Total mRNA was extracted from 50–100 mg tissue according to the instructions of the TRIzol kit (Invitrogen, Carlsbad, CA, USA). Total RNA yield was determined by measuring the absorbance of an aliquot of the precipitated stock at a wavelength of 260/280 nm to check for possible DNA contamination. After each extraction, samples were analyzed by reverse transcription (RT)-PCR without adding reverse transcriptase enzyme. BDNF, NGF, TrkB, and TrkA mRNA in each extraction were determined by real-time RT-PCR (Applied Biosystems, Foster City, CA, USA). GAPDH, used as an internal control, was co-amplified with BDNF, NGF, TrkB, and TrkA mRNAs. The primers were designed by AuGCT-Technology Company (Beijing, China) according to the serial number from Genebank as follows: BDNF: 5′-ATTAGGTGGCTTCATAGGAGAC-3′(sense)and 5′-GAACAGAACAGAACAGAACAGG-3′ (antisense); TrkB: 5′-TCTCTCGG TCTATGCCGTGGT GG-3′ (sense) and 5′-TCCAGGCACTTCCTCGT TCAGT-3′ (antisense); NGF: 5′-AGCGTAAT GTCCATGTTGTTCTAC-3′ (sense) and 5′-TGCT ATCTGTGTACGGTTCTGC-3′ (antisense); TrkA: 5′-CTTGCGCCGCATCCTGTCGT-3′ (sense) and

5′-GCAGGCCGCGGAGGGTATTC-3′ (antisense) and GAPDH: 5′-TTGCCATCAATGACCCCTTCA-3′ (sense) and 5′-CGCCCCACTTGATTTTGGA-3′ (antisense). Each primer contained comparable G/C content to minimize variability in hybridization efficiency at the annealing temperature.

The PCR mixture was amplified for 32 cycles with denaturation (94 °C, 15 s), annealing (60 °C, 30 s), and elongation (72 °C, 30 s) amplification steps. The reaction was terminated with a 5-min final elongation step (72 °C, 5 min). The PCR products were observed by electrophoresis on 1.5% agarose gel and the density of each band was analyzed on the gel image analysis system (Smartview 2001, S/N: SV-0002202). The level of the mRNA was determined by calculating the density ratio of each band of BDNF, TrkB, NGF and TrkA mRNA to GAPDH mRNA.

Statistical evaluation

The Statistical Package for the Social Science (SPSS) 15.0 was utilized for statistical analyses. All data are expressed as mean ± standard deviation (SD) of n subjects, and have been statistically analyzed with the Student's *t*-test, determined correlation-coefficient (r), and regression. *P* values less than 0.001 and 0.05 were considered statistically significant. Statistical evaluation through analysis of variance was also used to determine the demographic characteristics of suicide victims (n = 21) and non-psychiatric healthy control individuals (n = 19).

Results

Demographic characteristic of postmortem brain of suicide victims and non-psychiatric healthy control individuals

The demographic characteristics of suicide victims (n = 21) and non-psychiatric healthy control individuals (n = 19) was described earlier. The mean postmortem interval was 20.17 ± 5.4 h for control subjects and 21.45 ± 4.46 h for suicide victims. The brain pH for control participants was 6.19 ± 0.53 and for suicide victims was 6.54 ± 0.35. There were no significant differences in age ($F_{1,38} = 0.26$; t = 0.52; df = 38, $P > 0.05$), postmortem interval ($F_{1,38} = 0.67$; t = 0.82; df = 38, $P > 0.05$), or pH of the brain ($F_{1,38} = 3.48$; t = 1.86; df = 38, $P > 0.05$) between groups.

Quantitative analysis of BDNF and NGF in the hippocampus of suicide victims and non-psychiatric healthy control individuals

We quantitatively measured BDNF and NGF concentrations by sandwich ELISA in the hippocampus of suicidal victims and non-psychiatric healthy controls. Suicide victims showed significantly reduced BDNF concentration (t = 20.14; df = 19; $P < 0.001$; Fig. 1) compared to non-psychiatric healthy control individuals. Similarly, hippocampal NGF concentration was also significantly reduced (t = 13.39; df = 19; $P < 0.001$; Fig. 1) in suicide victims compared to non-psychiatric healthy control persons.

Expression of BDNF, NGF, TrkB, and TrkA protein in the hippocampus of suicide victims and non-psychiatric healthy control individuals

We analyzed expression profiles of both neurotrophins BDNF and NGF in the hippocampus by Western blotting and observed significantly reduced levels ($t_{BDNF} = 15.36$; df = 19; $P < 0.001$ and $t_{NGF} = 20.80$; df = 19; $P < 0.001$ respectively; Fig. 2) among suicide victims compared to non-psychiatric healthy controls. Similarly, reduced expression was also observed during the analysis of their receptor, that is $t_{TrkB} = 21.99$; df = 19; $P < 0.001$ and $t_{TrkA} = 22.23$; df = 19; $P < 0.001$ in Figure 2. The molecular weight of BDNF, NGF, TrkB, TrkA, and β-actin were 14, 13.5, 145, 140, and 42 kDa, respectively. Expression of BDNF, NGF, TrkB, and TrkA proteins were normalized against the internal control β-actin to determine their expression.

mRNA expression of BDNF, NGF, TrkB, and TrkA in the hippocampus of suicide victims and non-psychiatric healthy control individuals

We also measured mRNA expression of BDNF, NGF, TrkB, and TrkA that were significantly reduced ($t_{BDNF} = 9.31$; df = 19; $P < 0.001$; $t_{TrkB} = 28.25$; df = 19; $P < 0.001$; $t_{NGF} = 19.74$; df = 19; $P < 0.001$
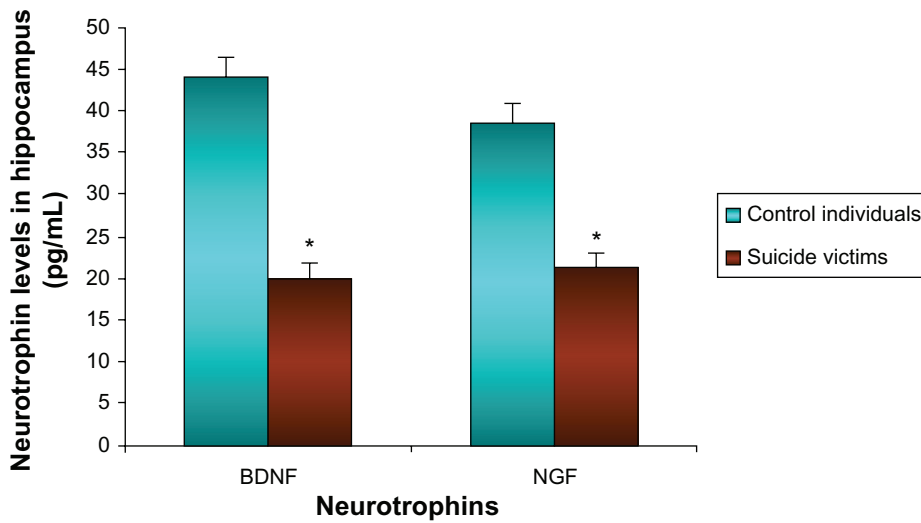
Figure 1. BDNF and NGF protein levels were significantly reduced among suicide victims compared to normal healthy individuals. Data are the mean ± SD and *$P < 0.001$.

and $t_{TrkA} = 26.23$; df = 19; $P < 0.001$; Fig. 3) in the hippocampus of participants who died by suicide and the differences were statistically significant. The lengths of BDNF, NGF, TrkB, TrkA, and GAPDH amplified fragments were 178, 199, 79,

96, and 173 base pairs, respectively. The levels of BDNF, NGF, TrkB, and TrkA mRNA were normalized against GAPDH mRNA levels as an internal control. GAPDH mRNA expression was not altered among the participants of both control and suicide
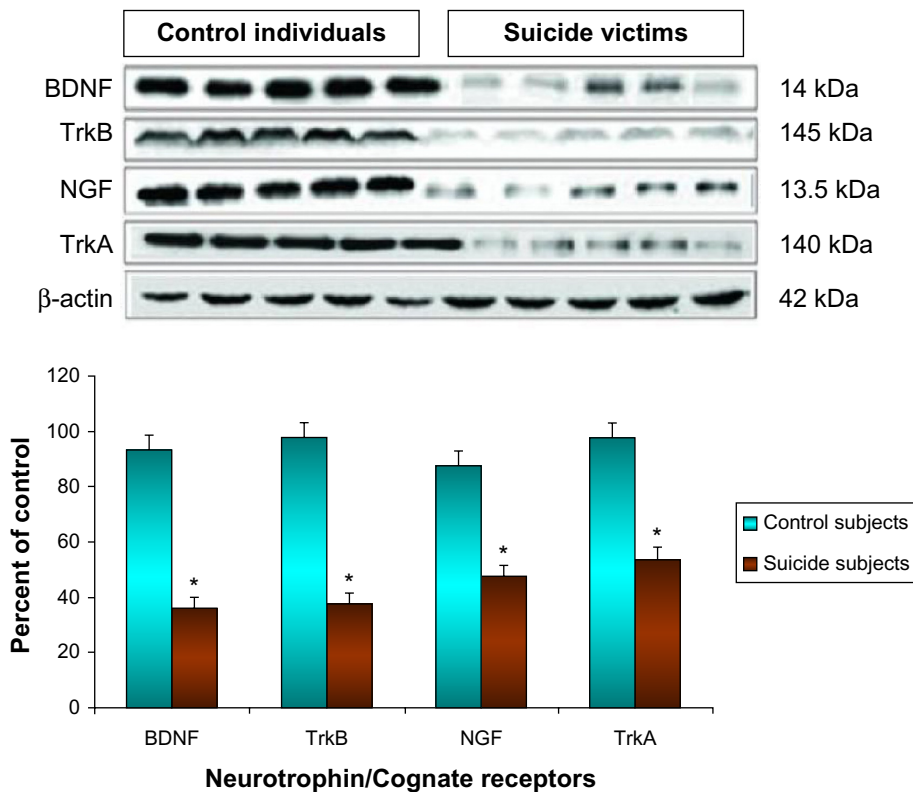

Figure 2. Representative bands of Western blot showing the protein levels of BDNF, TrkB, NGF, and TrkA in the hippocampus of suicide subjects and non-psychiatric healthy control subjects. Data are the mean ± SD and *$P < 0.001$. Hippocampus samples were from 19 non-psychiatric healthy control individuals and 21 suicide victims.
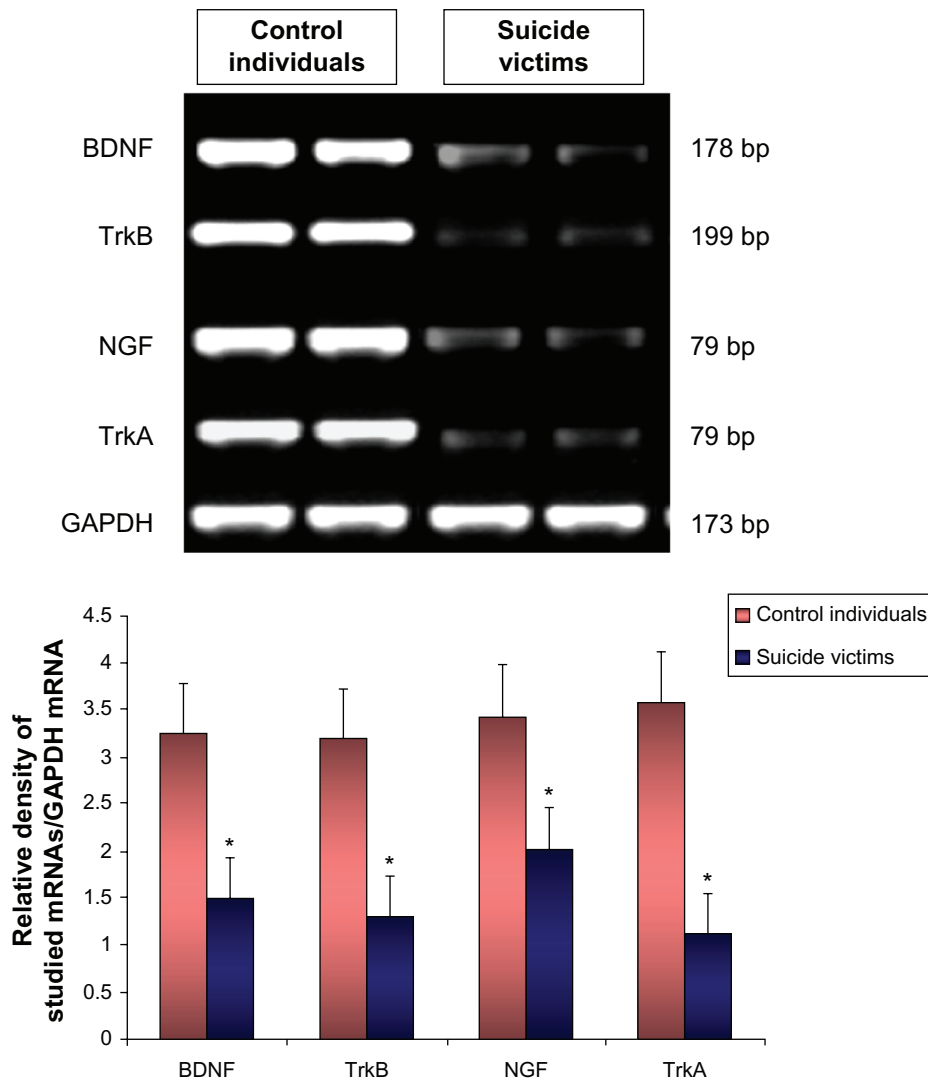
Figure 3. Representative mRNA levels of BDNF, TrkB, NGF, and TrkA in the hippocampus of suicide subjects and normal controls. Data are the mean ± SD and $*P < 0.001$. Hippocampus samples were from 19 normal controls and 21 suicide victims.

groups ($t_{GAPDH} = 0.100917$; df = 19; $P = 0.46$; Fig. 3).

Correlations between protein and mRNA levels of BDNF, TrkB, NGF, and TrkA in the hippocampus of postmortem brain of suicide victims

We also correlated the reduced expression profiles of neurotrophins and their respective receptors for protein and mRNA levels. BDNF and NGF protein levels were positively correlated with their respective mRNA levels ($r_{BDNF} = 0.923$; df = 20; $P < 0.05$ and $r_{NGF} = 0.848$; df = 20; $P < 0.05$, respectively). Significant positive correlations ($r_{TrkB} = 0.926$; df = 20; $P < 0.05$ and $r_{TrkA} = 0.831$; df = 20; $P < 0.05$,

respectively) between protein and mRNA levels were observed among their cognate receptors, TrkB and TrkA.

Discussion

Depression is a complex disorder that involves multiple neuronal substrates and brain regions. Among several alterations, neurotrophins regulation, particularly low levels of BDNF and NGF, have been suggested to lead to a depression-like state.

In the present study, quantitative evaluations of BDNF, NGF, and their cognate receptors TrkB and TrkA at both the protein and mRNA levels in the hippocampus of 21 participants who died by suicide and 19 non-psychiatric healthy controls were determined

by sandwich ELISA, Western blot, and real-time PCR. The main conclusions of our study are: (1) suicide subjects showed a statistically significant reduction of protein levels of BDNF, NGF, and their cognate receptors as investigated by ELISA and Western blot, respectively and (2) we observed a statistically significant decrease in translation of BDNF along with its receptor TrkB as well as NGF along with its receptor TrkA at the mRNA level.

Multiple lines of evidence indicate that BDNF is present at low levels in the blood cells and plasma of depressed patients. Postmortem suicide victims with or without depression and genetic association studies linking BDNF to suicide suggest that suicidal behavior may be associated with a decrease in BDNF function.[37,38] BDNF alone or in combination with other neurotransmitters is known to regulate synaptic plasticity, neurogenesis, and neuronal survival through its specific signaling pathways.[39,40] Additionally, stressful events such as a suicide attempt may alter the responsiveness of the HPA system and stress-induced elevation of glucocorticoids to reduce expression of BDNF in the blood and brain.[41] In the present study, hippocampal BDNF and TrkB contents were significantly reduced among individuals who died by suicide compared to non-psychiatric healthy individuals which agrees with the results of Dwivedi et al.[18] Similarly, when mRNA expression level was determined among these individuals, the mRNA levels of BDNF and TrkB were significantly reduced in the hippocampus of suicide victims compared to in non-psychiatric healthy control individuals. Such brain-derived neurotrophic factors exert their physiological effects by binding with TrkB receptors, which exist in truncated and full-length forms, both of which are important in mediating the functions of BDNF.[42,44]

NGF content may be reduced by changes in neuronal activity during stress. In support of this hypothesis, it has been demonstrated that expression of hippocampal NGF is dependent on neuronal activation and release of cholinergic neurotransmitters.[45,46] NGF may be important for counteracting the neurotoxic effects of glucocorticoids, which are elevated during stress.[47] The observed reduction in NGF after a stressful experience may be of pathophysiological relevance. NGF serves as a survival protein for cholinergic neurons; it can reverse cellular damage and reduce vulnerability to toxic influences.[48] Thus, a reduction in NGF content due to stress may have detrimental consequences for the survival of cholinergic neurons. Our study showing a significant reduction in hippocampal NGF and TrkA mRNA expression among the individuals who died by suicide compared to normal controls agree with the observations of Dwivedi et al.[18] The results of the present study indicate significant defective brain neurotrophin milieus in suicide victims, strengthening the role of neurotrophins in the pathophysiology of suicide.

Though the present findings are of particular relevance for understanding the neurochemical basis of suicide, it was beyond the scope of the present investigation conduct an in-depth investigation of the molecular mechanisms of these neurochemical alterations associated with stress. Future studies are needed to not only understand the molecular basis of the disease but also to design future therapeutic modalities targeting those molecular pathways.

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Author Contributions

Conceived and designed the experiments: RB, AKG, ACM. Analyzed the data: RB, BG, SB. Wrote the first draft of the manuscript: RB. Contributed to the writing of the manuscript: ACM, AKG. Agree with manuscript results and conclusions: BG, SB. Jointly developed the structure and arguments for the paper: RB, ACM. Made critical revisions and approved final version: RB, AKG, BG, SB, ACM. All authors reviewed and approved of the final manuscript.

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Competing Interests

Author(s) disclose no potential conflicts of interest.

Disclosures and Ethics

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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