ORIGINAL ARTICLE

Altered Gene Expression of Brain-Derived Neurotrophic Factor and Receptor Tyrosine Kinase B in Postmortem Brain of Suicide Subjects

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Background: Suicide is a major public health concern. Although authors of many studies have examined the neurobiological aspects of suicide, the molecular mechanisms associated with suicidal behavior remain unclear. Brain-derived neurotrophic factor (BDNF), one of the most important neurotrophins, after binding with and activating receptor tyrosine kinase B (trk B), is directly involved in many physiological functions in the brain, including cell survival and synaptic plasticity. The present study was performed to examine whether the expression of BDNF and/or trk B isoforms was altered in postmortem brain in subjects who commit suicide (hereafter referred to as suicide subjects) and whether these alterations were associated with specific psychopathologic conditions.

Methods: These studies were performed in prefrontal cortex in Brodmann area 9 and hippocampus obtained in 27 suicide subjects and 21 nonpsychiatric control subjects. Levels of messenger RNA and protein levels of BDNF and trk B were determined with competitive reverse transcriptase–polymerase chain reaction and Western blot technique, respectively. The level of neuron-specific enolase messenger RNA as a neuronal marker was also determined in these brain areas.

Results: Messenger RNA levels of BDNF and trk B were significantly reduced, independently and as a ratio to neuron-specific enolase, in both prefrontal cortex and hippocampus in suicide subjects, as compared with those in control subjects. These reductions were associated with significant decreases in the protein levels of BDNF and of full-length trk B but not trk B's truncated isoform. These changes were present in all suicide subjects regardless of psychiatric diagnosis and were unrelated to postmortem interval, age, sex, or pH of the brain.

Conclusions: Given the importance of BDNF in mediating physiological functions, including cell survival and synaptic plasticity, our findings of reduced expression of BDNF and trk B in postmortem brain in suicide subjects suggest that these molecules may play an important role in the pathophysiological aspects of suicidal behavior.

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SUICIDE IS a major public health concern. About 30 000 persons commit suicide each year in the United States, and it is a frequent outcome of major psychiatric disorders.[1] Results of several studies suggest that the lifetime suicide attempt rate among adults is about 10%.[2] Despite the devastating effect of suicide on numerous lives, there is still a dearth of knowledge concerning its underlying cause and pathologic mechanism.

Authors of several clinical and epidemiological studies have identified stress as an important risk factor in suicide.[3,4] Furthermore, autopsy studies have shown a high rate of affective disorders as 1 of the main causes of increased mortality among those who commit suicide.[5] Emerging studies suggest that stress and affective disorders may both be associated with structural abnormalities in the brain. For example, patients with affective disorders have reduced hippocampal volume, reduced density and size of cortical neurons in the dorsolateral prefrontal cortex and orbitofrontal cortex, reduced density of nonpyramidal neurons, layer-specific reduction in interneurons of the anterior cingulate cortex and in nonpyramidal neurons of the hippocampal formation, and a marked decrease in glial cell number and density in the prefrontal cortex.[6-8] On the other hand, authors of several studies show that stress or glucocorticoid administration causes neuronal atrophy,[9-11] a decrease in the number or length of apical dendrites,[12] and even loss of hippocampal neurons[13] in rodents or nonhuman primates. Results in a few studies directly demonstrate that the size of the parahippocampal cortex[14] and cortical

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laminar thickness[15] are reduced in those who commit suicide. Taken together, the results of these studies indicate that suicide could be associated with atrophy or loss of neurons and/or glia in the brain.

Neurotrophins are one of the epigenetic factors that may influence the development and survival of neurons in the central nervous system. One of the most important and widely distributed members of the neurotrophin family in the brain is brain-derived neurotrophic factor (BDNF).[16] After binding and activating a specific receptor, that is, receptor tyrosine kinase B (trk B), BDNF is directly involved in neurite outgrowth, phenotypic maturation, morphological plasticity, and synthesis of proteins for differentiated functioning of neurons and synaptic functioning.[16] Results of recent studies suggest that BDNF is also involved in nerve regeneration, structural integrity, and maintenance of neuronal plasticity in the adult brain.[17-19] A pathologic alteration of the neurotrophic factor system thus may not only lead to defects in neural maintenance and regeneration, and therefore structural abnormalities in the brain, but also reduce neural plasticity and therefore impair the individual's ability to adapt to crisis situations.

Results of many preclinical studies demonstrate that several types of stressors regulate the expression of BDNF in the brain.[20-22] In addition, preclinical and clinical study results indicate that BDNF could be involved in depression and in the mechanism of action of antidepressants.[23-28] We recently observed that expression and functional characteristics of transcription factor cyclic adenosine monophosphate response element binding protein (CREB), which is involved in the regulation of many neuronally expressed genes, including BDNF, is severely reduced in postmortem brain in those who commit suicide.[29]

Given the importance of BDNF in maintenance of the structural integrity and synaptic plasticity of the brain, and its involvement in stress and affective disorders, we investigated the potential role of BDNF in suicidal behavior by examining the expression of BDNF in postmortem brain in subjects who committed suicide (hereafter referred to as suicide subjects) and nonpsychiatric control subjects. Since BDNF mediates its biological action after binding to trk B, which exists as truncated and full-length isoforms, both of which are functionally important in mediating BDNF-induced biological functions,[30-32] we determined the expression of each trk B isoform separately. In addition, we examined whether the changes in expression of BDNF and/or trk B were present in all suicide subjects irrespective of psychiatric diagnosis or if these changes were associated with major depression.

METHODS

SUBJECTS

Brain tissue was collected by the Brain Collection Program of the Maryland Psychiatric Research Center, Baltimore, in collaboration with the Medical Examiner's Office of the State of Maryland. Brain samples were free of neuropathologic abnormalities or human immunodeficiency virus antibodies. Toxicological data were obtained by analysis of urine and blood samples.

Psychiatric diagnoses in suicide subjects and control subjects were evaluated with the Diagnostic Evaluation After Death[33] and the Structured Clinical Interview for *DSM-IV*.[34] Family members gave permission for clinical records to be obtained from mental health treatment providers when there was a prior history of mental health treatment and in all cases of suicide. The present studies were performed in the prefrontal cortex in Brodmann area 9 and in hippocampus obtained from the right hemisphere of the brain in 27 suicide subjects and 21 nonpsychiatric control subjects. The prefrontal cortex was defined as the gray matter from the most anterior 1-cm coronal slice of the cortex and was further dissected according to the Brodmann atlas. The hippocampus was 0.5-cm coronal slices of the middle of the hippocampus, which included the dentate gyrus and areas CA1-4. This study was approved by the institutional review boards of the University of Maryland, Baltimore, and of the University of Illinois at Chicago.

QUANTITATION OF BDNF AND trk B WITH WESTERN BLOT TECHNIQUE

BDNF and full-length and truncated trk B were immunolabeled with the Western blot technique as described by Ferrer et al.[35] Equal volumes of soluble fractions containing 70 µg of protein were electrophoresed on 15% (weight-volume ratio) polyacrylamide gel. The blots were incubated overnight at 4°C with primary antibody for BDNF (1:1000 dilution; R&D Systems Inc, Minneapolis, Minn) or full-length or truncated trk B (1:500 dilution; Santa Cruz Biotechnology Inc, Santa Cruz, Calif). The membranes were stripped and reprobed with β-actin monoclonal antibody (1:5000 for 1 hour; Sigma-Aldrich Corp, St Louis, Mo). The bands on the autoradiographs were quantified. The optical density of each protein was corrected by using the optical density of the corresponding β-actin band.

To determine the specificity of antihuman BDNF antibody, we used SH-SY5Y whole-cell lysate (Santa Cruz Biotechnology Inc) and recombinant human BDNF (Amgen Inc, Thousand Oaks, Calif). Thirty µg of protein was subjected to 15% polyacrylamide gel electrophoresis as described earlier. Specificity of full-length and truncated trk B was confirmed by means of preincubating the antibodies with antigenic peptides corresponding to full-length or truncated trk B (Santa Cruz Biotechnology Inc).

DETERMINATION OF MESSENGER RNA LEVELS OF BDNF, trk B, AND NEURON-SPECIFIC ENOLASE

Total RNA was isolated by using cesium chloride ultracentrifugation as described previously.[36] The degradation of messenger RNA (mRNA) was assessed by using denaturing agarose gel electrophoresis and evaluating the sharpness of 28S and 18S ribosomal RNA bands.

BDNF, trk B, and neuron-specific enolase (NSE) mRNAs were quantified by using internal standards.[37] Primer pairs 5′ AAG GAC GCA GAC TTG TAC ACG (forward) and 5′ CAT GGG ATT GCA CTT GGT CTC (reverse) were designed to allow amplification of 253-567 base pairs (bp) for BDNF (GenBank accession number X91251), primer pairs 5′ ATC TGG CCG CAC CTA ACC TCA (forward) and 5′ TAT TGC CCC GTT ATA GAA CCA (reverse) for amplification of 599-969 bp for trk B (GenBank accession number S576473), and primer pairs 5′ GGG ACT GAG AAC AAA TCC AAG (forward) and 5′ CTC CAA GGC TTC ACT GTT CTC (reverse) for amplification of 295-675 bp for NSE (GenBank accession number X14327). The internal primer sequences for BDNF, trk B, and

Table 1. Characteristics of Suicide and Control Subjects

Subject No./ Age, y/ Race/Sex	PMI, h	Brain pH	Cause of Death	Toxicologic Findings at Time of Death	Psychiatric Diagnosis
				Suicide Subjects*	
1/22/B/F	16	5.3	Drug overdose	Propranolol hydrochloride	Major depression
2/24/W/M	7	5.6	GSW	None	Major depression
3/21/W/M	17	6.1	GSW	None	Major depression, adjustment disorder
4/27/W/M	24	6.4	GSW	None	Major depression, alcohol abuse
5/38/W/M	24	6.3	Drug overdose	Alcohol, diphenhydramine hydrochloride	Major depression, alcohol abuse
6/36/W/F	10	6.5	GSW	Butalbital, diphenhydramine hydrochloride, acetaminophen, amitriptyline hydrochloride, desipramine hydrochloride	Major depression
7/41/W/F	27	5.9	Drug overdose	Diphenhydramine hydrochloride, nortriptyline hydrochloride, pseudophedrine hydrochloride, salicylate meglumine, alcohol	Major depression, alcohol abuse
8/44/W/F	11	5.6	Drug overdose	Nortriptyline hydrochloride	Major depression, alcohol abuse
9/46/W/F	16	6.1	Drug overdose	Nortriptyline hydrochloride	Major depression, agoraphobia
10/46/W/F	21	5.3	Drug overdose	Amitriptyline hydrochloride, desipramine hydrochloride, alcohol	Major depression
11/53/W/M	23	6.1	Jumped	None	Major depression
12/24/W/M	22	6.5	Hanging	None	Schizoaffective disorder
13/40/W/M	26	5.6	GSW	Alcohol	Adjustment disorder
14/68/W/F	26	6.1	GSW	None	Schizoaffective disorder
15/37/B/M	NA	5.8	Carbon monoxide intoxication	Carbon monoxide	NA
16/26/B/M	NA	6.5	Hanging	Cocaine	NA
17/50/W/M	7	6.1	GSW	None	No psychiatric illness
18/24/B/M	22	6.6	GSW	None	No psychiatric illness
19/75/W/M	18	6.7	GSW	None	Adjustment disorder, conduct disorder
20/40/W/M	17	6.2	Jumped	None	Schizophrenia
21/41/B/M	12	6.3	Multiple injuries	None	No psychiatric illness
22/87/W/M	16	6.2	GSW	None	Adjustment disorder
23/43/W/M	12	6.5	Drug overdose	Acetaminophen, propoxyphene hydrochloride	Polysubstance abuse
24/34/W/M	16	6.2	GSW	Alcohol	Alcohol abuse
25/39/W/M	30	6.5	Asphyxia	Freon, cocaine metabolite	Drug abuse
26/30/W/M	32	6.4	Hanging	Cocaine, alcohol	Drug and alcohol abuse
27/51/W/F	28	6.7	Drug overdose	Amitriptyline hydrochloride, alcohol	Bipolar disorder
				Control Subjects†	
28/45/W/M	22	6.5	ASCVD	None	. . .
29/22/B/M	19	6.2	GSW	None	. . .
30/83/W/M	20	5.6	ASCVD	None	. . .
31/63/W/F	30	5.7	Ovarian cancer	None	. . .
32/31/B/M	8	5.6	GSW	None	. . .
33/35/W/M	24	5.6	Crash injury	None	. . .
34/33/W/M	15	6.0	GSW	Acetaminophen	. . .
35/37/B/M	5	6.6	ASCVD	None	. . .
36/37/W/M	24	6.3	ASCVD	None	. . .
37/65/B/F	23	5.6	ASCVD	None	. . .
38/38/B/M	16	5.8	Lung sarcoidosis	None	. . .
39/40/W/F	7	6.5	ASCVD	None	. . .
40/23/B/M	15	6.7	GSW	None	. . .
41/42/W/F	23	6.2	Pneumonia	None	. . .
42/46/B/M	9	6.2	Multiple injuries	None	. . .
43/48/W/M	26	6.1	ASCVD	None	. . .
44/52/W/M	30	6.3	ASCVD	None	. . .
45/37/B/M	9.5	6.1	ASCVD	None	. . .
46/43/W/M	17.5	5.7	NA	None	. . .
47/41/W/M	24	6.2	NA	None	. . .
48/41/B/M‡	27	5.8	Liver cirrhosis	None	. . .

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; GSW, gunshot wound; NA, not available; PMI, postmortem interval.
*Mean ± SD age was 41.0 ± 16.0 years; PMI, 19.2 ± 7.0 hours; and brain pH, 6.1 ± 0.4. Five black and 22 white subjects; 8 female and 19 male subjects.
†Mean ± SD age was 42.9 ± 13.9 years; PMI, 18.7 ± 7.7 hours; and brain pH, 6.1 ± 0.3. Twelve white and 9 black subjects; 4 female and 17 male subjects.
‡Hippocampus was not available.

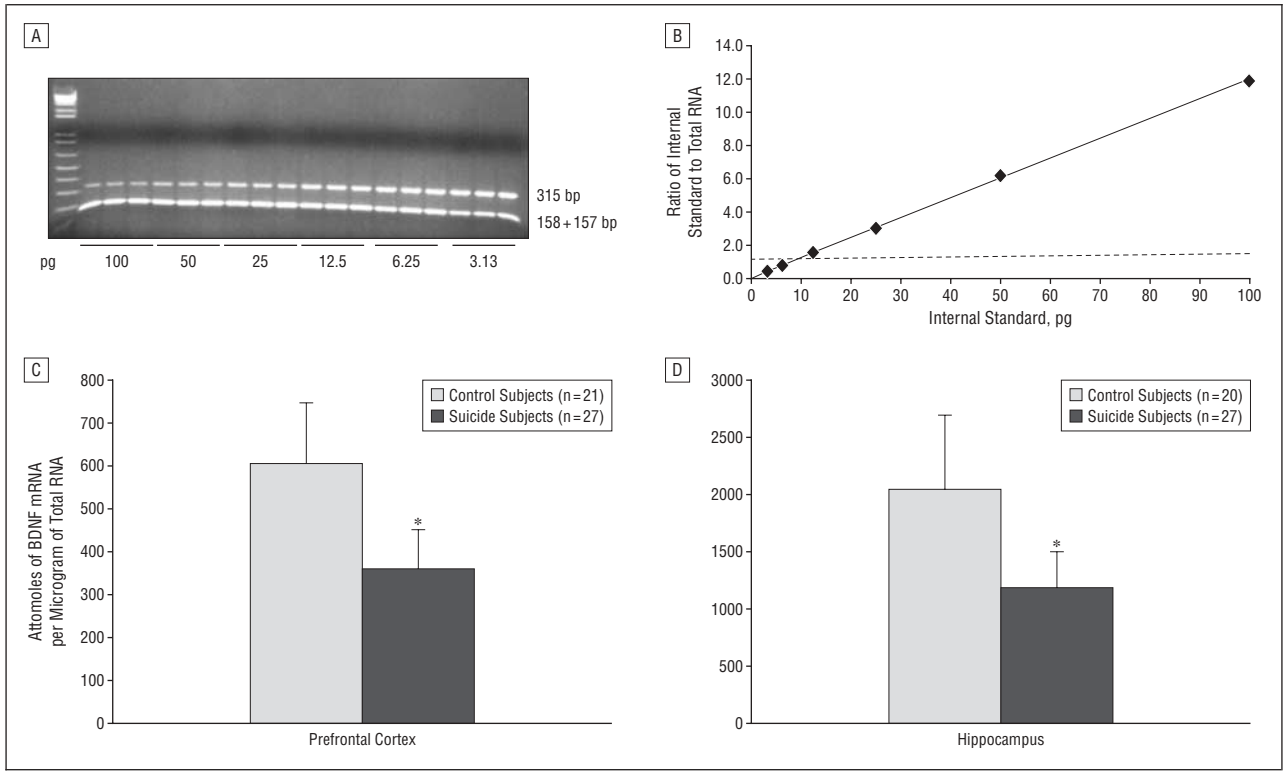

Figure 1. A, Representative agarose gel electrophoresis showing a competitive reverse transcriptase–polymerase chain reaction (RT-PCR) for brain-derived neurotrophic factor (BDNF) messenger RNA (mRNA) in prefrontal cortex obtained in 1 control subject. B, Competitive RT-PCR analysis of BDNF mRNA content. The data derived from agarose gel electrophoresis were plotted as the counts incorporated into the amplified complementary RNA standard divided by the counts incorporated into the BDNF mRNA amplification product vs the known amount of internal standard. The point of equivalence represents the amount of BDNF mRNA. The mean±SD of BDNF mRNA in prefrontal cortex (C) and hippocampus (D) in control subjects and suicide subjects. Suicide group was compared with control group (cortex, t_{45}=10.58, P<.001 [95% confidence interval, 173.64-317.98]; hippocampus, t_{46}=10.18, P<.001 [95% confidence interval, 550.56-1193.64]). Error bars indicate SD. Asterisks indicate P<.001; bp, base pair. In 1 control subject, hippocampus was not available.

NSE were 5′ CGA GG*A* GA*T* CTG AGC GTG TGT (403-423 bp), 5′ AAG CAG AT*C* TC*G* A*G*T GTG GCG GAA (784-807 bp), and 5′ GGC AA*C* AAG CT*C* G*AG* ATG CAG GAG TTC (478-504 bp), respectively. The underlined bases indicate the *Bgl*II (BDNF) and *Xho*I (trk B and NSE) restriction sites, whereas boldface italicized bases indicate the mutation sites. To quantify mRNA levels, we added decreasing concentrations of BDNF (100-3.125 pg), trk B (400-12.5 pg), or NSE (50-3.125 pg) internal standard complementary RNAs to 1 µg of total RNA. The polymerase chain reaction (PCR) mixture was amplified for 32 cycles. After amplification, aliquots were digested with *Bgl*II (BDNF) or *Xho*I (trk B and NSE) and run by means of 1.5% agarose gel electrophoresis. Amplification of the PCR mixture at 20 to 40 cycles showed that 32-cycle amplification was in the linear range.

STATISTICAL ANALYSIS

Data were analyzed with statistical software (SPSS 8.0; SPSS, Chicago, Ill). All values are reported as mean±SD. The differences in mRNA and protein levels of BDNF or trk B and age, sex, and postmortem interval (PMI) between suicide subjects and control subjects were analyzed by using the independent-sample *t* test. The relationships among mRNA and protein levels of BDNF or trk B and PMI, age, pH of brain, and sex were determined by using Pearson product moment correlation analysis. *P* values were 2 tailed. The statistical differences in levels of BDNF or trk B between the subgroups of suicide subjects and control subjects were evaluated by using 1-way analysis of variance. During data analysis, we included race as a confounding variable. Multiple comparisons were conducted, and we report unadjusted *P* values. We have indicated in the tables the Bonferroni-adjusted α level and the rationale for its adjustment.

RESULTS

Detailed demographic characteristics of suicide subjects and control subjects are provided in **Table 1**. There were 17 men and 4 women in the control group and 19 men and 8 women in the suicide group. The age range was 21 to 87 years, and the PMI was between 5 and 32 hours. There were no significant differences between suicide subjects and control subjects in age (t_{46}=0.44, P=.66) or PMI (t_{44}=0.21, P=.83). The mean brain pH in control subjects was 6.1±0.3 and in suicide subjects was 6.1±0.4, which was not significantly different (t_{46}=0.68, P=.49).

MESSENGER RNA LEVEL OF BDNF

Figure 1A shows findings of a representative gel electrophoresis of the competitive reverse transcriptase–PCR (RT-PCR) of BDNF mRNA in prefrontal cortex from 1 control subject. As expected, we found the amplification product arising from the BDNF mRNA template at 315 bp and the corresponding digestion products from the complementary RNA at 158+157 bp. Figure 1B presents the results of a competitive RT-PCR analysis, where the point of equivalence represents the absolute amount

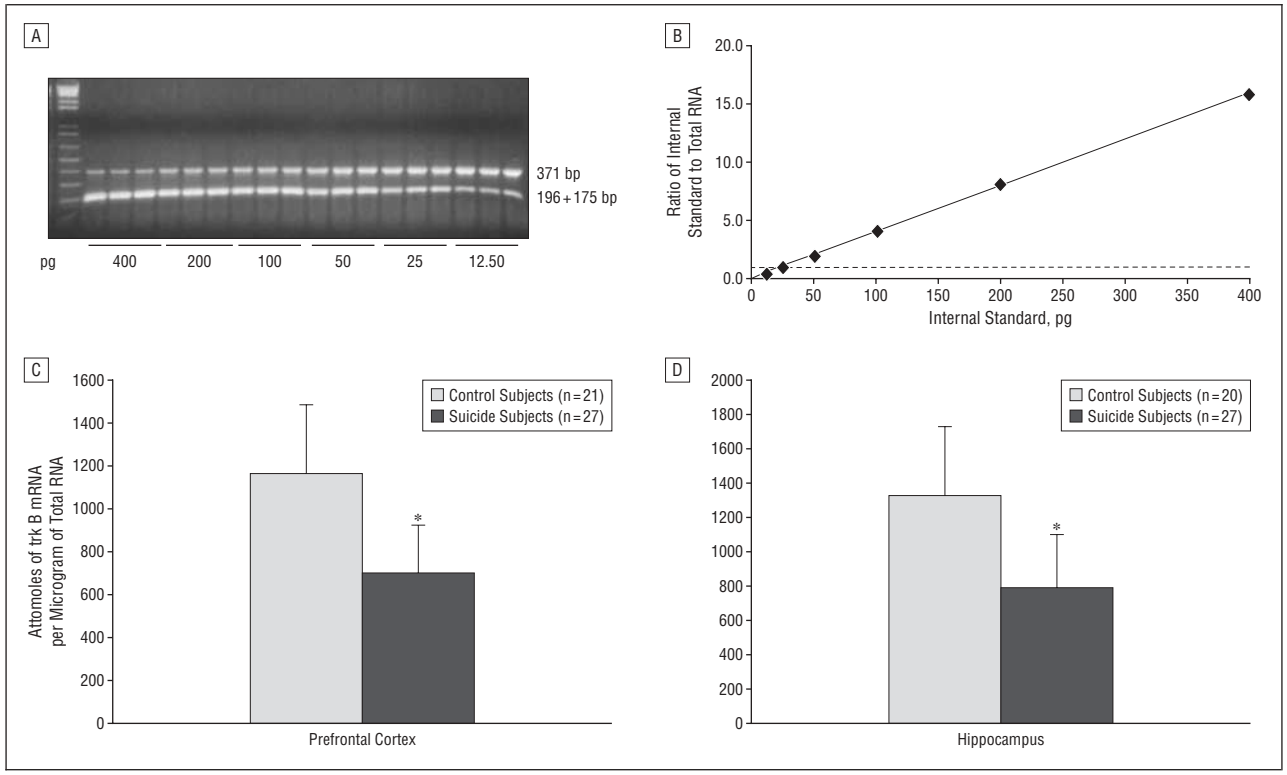

Figure 2. A, Representative agarose gel electrophoresis showing a competitive reverse transcriptase–polymerase chain reaction (RT-PCR) for receptor tyrosine kinase B (trk B) messenger RNA (mRNA) in prefrontal cortex obtained in 1 control subject. B, Competitive RT-PCR analysis of trk B mRNA content. The data derived from agarose gel electrophoresis were plotted as the counts incorporated into the amplified complementary RNA standard divided by the counts incorporated into the trk B mRNA amplification product vs the known amount of internal standard. The point of equivalence represents the amount of trk B mRNA. C and D, the mean±SD of trk B mRNA in prefrontal cortex and hippocampus, respectively, in control subjects and suicide subjects. Suicide group was compared with control group (cortex, t_{46}=12.9, $P<.001$ [95% confidence interval, 302.49-638.17]; hippocampus, t_{45}=9.56, $P<.001$ [95% confidence interval, 317.82-754.77]). Error bars indicate SD. Asterisks indicate $P<.001$. In 1 control subject, hippocampus was not available. bp indicates base pair.

of BDNF present. The expression of BDNF was higher in the hippocampus (Figure 1D) than in the prefrontal cortex (Figure 1C). Comparison revealed that the BDNF mRNA level was significantly lower in both prefrontal cortex (Figure 1C) and hippocampus (Figure 1D) in suicide subjects, as compared with that in control subjects.

MESSENGER RNA LEVEL OF trk B

Figure 2A shows findings of a representative gel electrophoresis of the competitive RT-PCR, and Figure 2B shows the competitive RT-PCR analysis for trk B mRNA in prefrontal cortex from 1 control subject. The amplification product for trk B arose from the trk B mRNA template at 371 bp and the corresponding digestion products from complementary RNA at 196+175 bp. Comparison showed that trk B mRNA was significantly lower in prefrontal cortex (Figure 2C) and hippocampus (Figure 2D) in suicide subjects, as compared with that in control subjects.

RATIOS OF NSE mRNA TO BDNF AND TO trk B mRNA

To establish whether neuronal RNA contributed equally to the total RNA pool, we determined the mRNA level of NSE in both prefrontal cortex and hippocampus in control subjects and suicide subjects. **Figure 3**A shows findings of a representative competitive RT-PCR gel electrophoresis, and Figure 3B shows the RT-PCR analysis for NSE. The amplification product of NSE mRNA arose at 381 bp, and after digestion with restriction enzymes it generated fragments of 195+186 bp. No significant differences in mRNA levels of NSE between control subjects and suicide subjects were observed in either prefrontal cortex (Figure 3C) or hippocampus (Figure 3D).

The ratios of BDNF and of trk B to NSE mRNA in prefrontal cortex and hippocampus are shown in **Figure 4**A and B, respectively. Messenger RNA levels of BDNF and trk B were still significantly lower in both prefrontal cortex and hippocampus in suicide subjects when expressed as a function of the respective NSE mRNA content to correct for nonspecific loss of mRNA owing to putative neuronal damage.

IMMUNOLABELING OF BDNF

The specificity of the BDNF antibody was checked with SH-SY5Y cell extract and recombinant human BDNF. As expected, the antibody showed a 14-kilodation (kDa) band in the cellular extract[23] and in the soluble fractions of prefrontal cortex and hippocampus (**Figure** 5A). Representative Western blots showing expressed levels of BDNF in prefrontal cortex from 3 control subjects and 3 suicide subjects are shown in Figure 5B. The immunolabeling of BDNF was significantly lower in both prefrontal

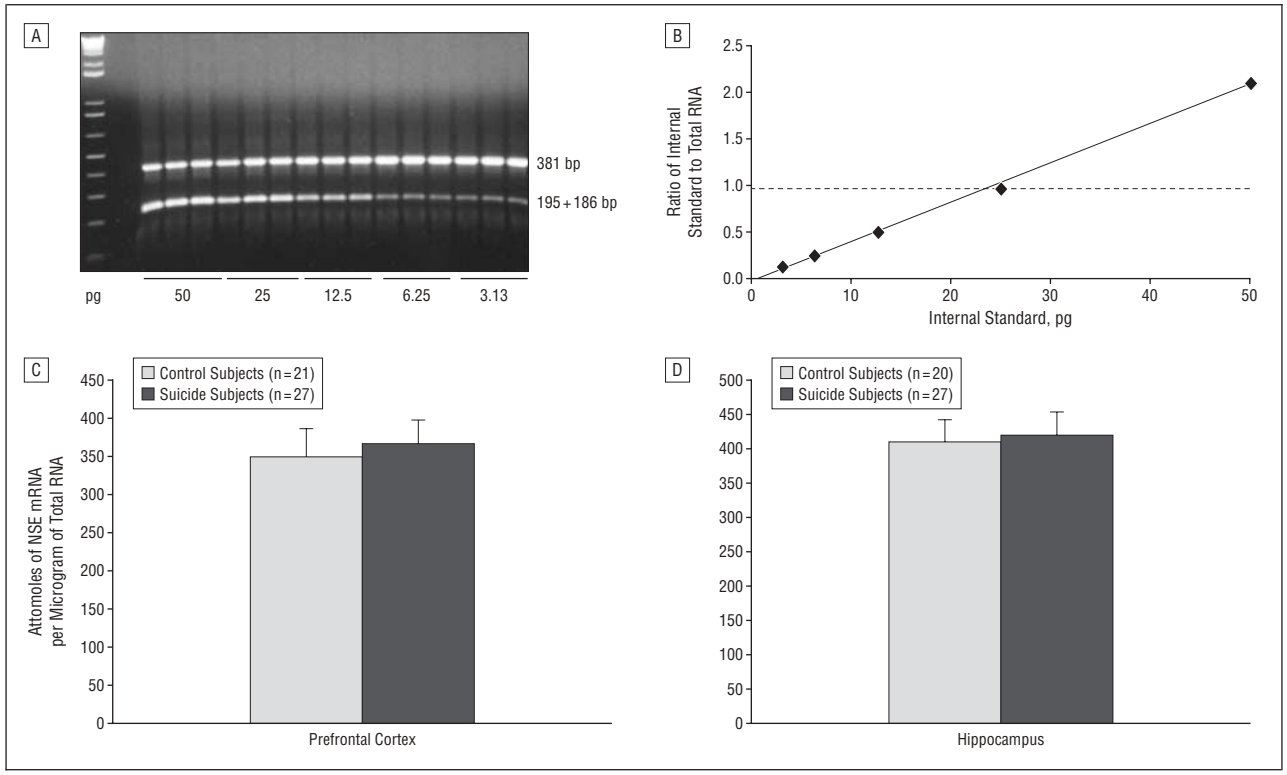

Figure 3. A, Representative agarose gel electrophoresis showing a competitive reverse transcriptase–polymerase chain reaction (RT-PCR) for neuron-specific enolase (NSE) messenger RNA (mRNA) in prefrontal cortex obtained in 1 control subject. B, Competitive RT-PCR analysis of NSE mRNA content. The data derived from agarose gel electrophoresis were plotted as the counts incorporated into the amplified complementary RNA standard divided by the counts incorporated into the NSE mRNA amplification product vs the known amount of internal standard. The point of equivalence represents the amount of NSE mRNA. C and D, the mean ± SD of NSE mRNA in prefrontal cortex and hippocampus, respectively, in control subjects and suicide subjects. Suicide group was compared with control group (cortex, t_{46}=1.71, P=.094 [95% confidence interval, -38.21 to 3.09]; hippocampus, t_{45}=1.00, P=.32 [95% confidence interval, -30.7 to 10.33]). Error bars indicate SD. In 1 control subject, hippocampus was not available. bp indicates base pair.

cortex (**Figure 6**A) and hippocampus (Figure 6B) in suicide subjects, as compared with that in control subjects.

IMMUNOLABELING OF FULL-LENGTH AND TRUNCATED trk B

Because the primer sequences for trk B do not distinguish between full-length and truncated trk B and both of these transcripts are functionally different, we examined the immunolabeling of full-length and truncated trk B separately. Representative Western blots of full-length and truncated trk B in prefrontal cortex in 3 suicide subjects and 3 control subjects are shown in **Figure 7**. Full-length trk B migrated to 145 kDa, whereas truncated trk B migrated to 95 kDa. Specificity of full-length and truncated trk B was checked by adding antigenic peptides corresponding to full-length and truncated trk B. In both prefrontal cortex and hippocampus, immunolabeling of full-length trk B was significantly lower in suicide subjects than in control subjects (**Figure 8**A), whereas immunolabeling of truncated trk B showed no significant difference between suicide subjects and control subjects (Figure 8B).

CORRELATIONAL ANALYSES OF mRNA AND PROTEIN LEVELS OF BDNF AND trk B

To examine the interrelationships among the lower mRNA and protein levels of BDNF with the decrease in mRNA expression of trk B and the protein expression of full-length trk B, we correlated the mRNA levels of BDNF and trk B with their respective protein levels. We observed a significant correlation between BDNF mRNA and protein (cortex, r=0.55 [$P < .001$]; hippocampus, r=0.43 [P=.002]) and between trk B mRNA and the full-length trk B protein (cortex, r=0.43 [P=.002]; hippocampus, r=0.50 [$P < .001$]).

EFFECTS OF POTENTIAL CONFOUNDING VARIABLES

The effects of confounding variables such as age, PMI, sex, and brain pH were evaluated with respect to the mRNA and protein levels of BDNF and of trk B. None of the confounding variables had any significant effects on mRNA or protein levels of BDNF or of trk B (data not shown).

EFFECTS OF DIAGNOSIS

To examine whether the differences in mRNA and protein levels of BDNF and trk B between control subjects and suicide subjects were related to depression, we placed the suicide subjects into categories: those with a diagnosis of major depression that was active at the time of death and those with diagnoses of other psychiatric disorders or of no mental illness. Of 27 suicide subjects, 11

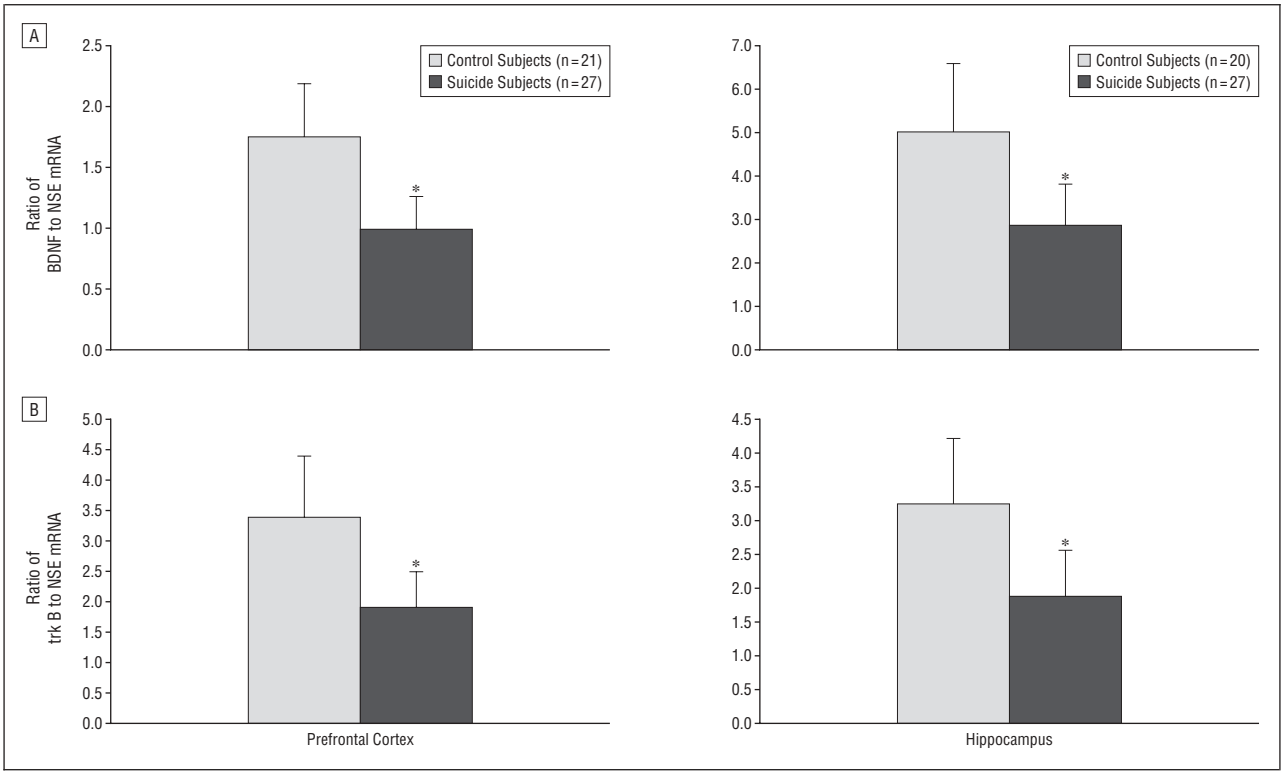

Figure 4. Ratios of brain-derived neurotrophic factor (BDNF) to neuron-specific enolase (NSE) messenger RNA (mRNA) (A) and receptor tyrosine kinase B (trk B) to NSE mRNA (B) in prefrontal cortex and hippocampus in control subjects and suicide subjects. The data are the mean ± SD. Suicide group was compared with control group. The significance levels were as follows: for BDNF/NSE: cortex, t_{46}=10.48, P<.001 (95% confidence interval [CI], 0.54-0.98); hippocampus, t_{46}=10.48, P<.001 (95% CI, 1.35-2.98) and for trk B/NSE: cortex, t_{46}=10.71, P<.001 (95% CI, 0.96-1.99); hippocampus, t_{46}=10.71, P<.001 (95% CI, 0.83-1.89). Error bars indicate SD. Asterisks indicate P< .001. In 1 control subject, hippocampus was not available.

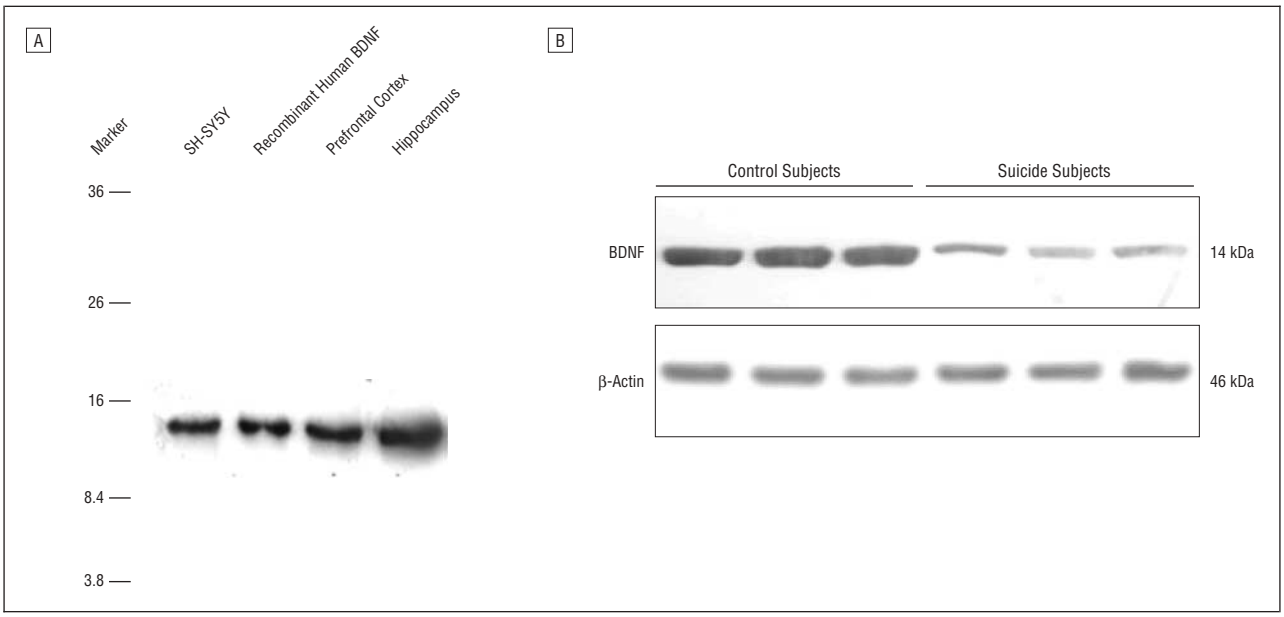

Figure 5. A, Representative Western blots showing the immunolabeling of brain-derived neurotrophic factor (BDNF) in SH-SY5Y cell extract, recombinant human BDNF, and soluble fractions of prefrontal cortex and hippocampus in 1 control subject. B, Representative Western blots showing the immunolabeling of BDNF and β-actin in prefrontal cortex in 3 control subjects and 3 suicide subjects. kDa indicates kilodalton.

had a diagnosis of major depression; 3, adjustment and/or conduct disorders; 1, schizoaffective disorder; 4, no psychiatric illness; 1, bipolar disorder; 2, alcohol abuse; 2, polysubstance abuse; and 1, schizophrenia. For 2 suicide subjects, no diagnosis was available. Messenger RNA and protein levels of BDNF, mRNA levels of trk B, and protein levels of full-length trk B did not differ between suicide subjects with major depression and suicide subjects with other psychiatric disorders. However, both groups showed significant differences when compared

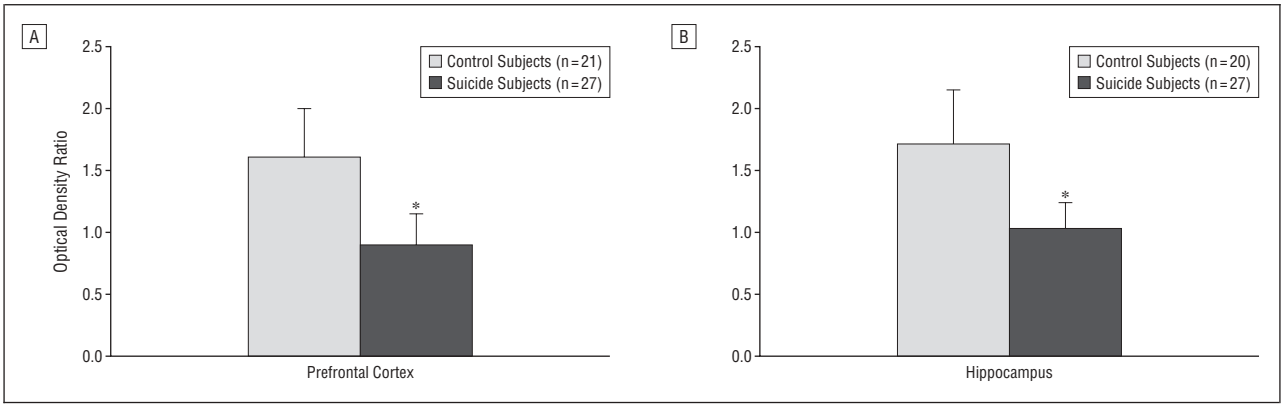

Figure 6. Immunolabeling of brain-derived neurotrophic factor (BDNF) . Mean±SD of the optical density ratio of BDNF to β-actin in prefrontal cortex (A) and hippocampus (B) in control subjects and suicide subjects. Suicide group was compared with control group (cortex, t_{46}=7.36, P<.001 [95% confidence interval, 0.50-0.91]; hippocampus, t_{45}=7.52, P<.001 [95% confidence interval, 0.45-0.89]). Error bars indicate SD. Asterisks indicate P< .001. In 1 control subject, hippocampus was not available.

with control subjects for both prefrontal cortex (**Table 2**) and hippocampus (**Table 3**).

EFFECTS OF ANTIDEPRESSANT TOXICITY

To examine whether the observed changes in BDNF and trk B expression in the suicide group were related to antidepressant toxicity, we placed suicide subjects into 2 categories—those who showed effects of antidepressant toxicity at the time of death (n=7) and those who did not (n=20). We did not find significant differences in mRNA (cortex, 357.12±120.92 vs 362.63±85.45 attomoles per microgram of total RNA; hippocampus, 1205.25±193.13 vs 1165.52±365.63 attomoles per microgram of total RNA) or protein (cortex, 1.07±0.28 vs 0.84±0.22 arbitrary units; hippocampus, 1.15±0.10 vs 0.98±0.23 arbitrary units) levels of BDNF between suicide subjects who showed effects of antidepressant toxicity at the time of death and those who did not. Similarly, no significant differences in mRNA (cortex, 725.12±175.14 vs 686.57±246.51 attomoles per microgram of total RNA; hippocampus, 712.62±260.28 vs 821.37±326.01 attomoles per microgram of total RNA) or protein (cortex, 0.86±0.24 vs 0.86±0.18 arbitrary units; hippocampus, 0.92±0.21 vs 0.90±0.36 arbitrary units) levels of trk B were observed between these 2 groups.

Of 11 depressed suicide subjects, only 2 (subjects 10, 11) were being treated with tricyclic antidepressants 1 month before death; 4 suicide subjects (subjects 6-9) were treated with tricyclic antidepressants 3 or 6 months before death. Comparison of suicide subjects who were depressed and were treated with antidepressants vs those who were untreated revealed no significant differences in levels of BDNF or trk B (data not shown).

COMMENT

To our knowledge, this study is the first in which expression levels of BDNF and trk B in postmortem brain in suicide subjects is examined. We found significantly lower levels in mRNA and protein expression of both BDNF and trk B in the prefrontal cortex and hippocampus in suicide subjects, which were present in all suicide subjects, regardless of psychiatric diagnosis. Our study results suggest that suicidal behavior could be associated with abnormalities in BDNF and its mediated functions.

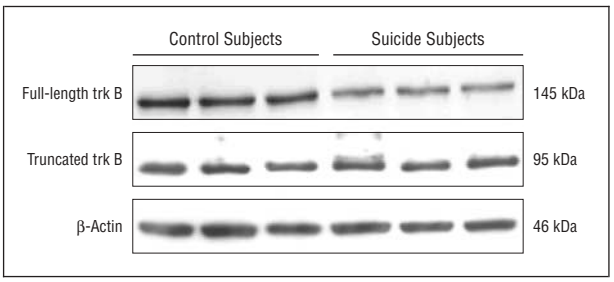

Figure 7. Representative Western blots of full-length and truncated receptor tyrosine kinase B (trk B) and β-actin in prefrontal cortex in 3 control subjects and 3 suicide subjects. kDa indicates kilodalton.

Authors of several studies provide evidence that BDNF could be involved in depressive behavior. This evidence stems mainly from observations that long-term but not short-term treatment with antidepressants or electroconvulsive shock increases the expression of BDNF in rat brain.[24,25] Furthermore, recent study results demonstrated that BDNF immunoreactivity is increased in postmortem brain in patients with depression treated with antidepressants.[23] More direct evidence for the role of BDNF in depression comes from recent study results showing that the serum level of BDNF is lower in patients with major depression.[28] In our present study, we found that the expression of BDNF in postmortem brain was lower not only in suicide subjects with depression but also in suicide subjects with other psychiatric disorders, which suggests that decreased expression of BDNF may not be specific to depression but could be associated with suicidal behavior. In contrast to the findings that antidepressant treatment causes an increase in expression of BDNF, our study results show no significant effects of antidepressants on BDNF level. However, given the small number of suicide subjects with depression treated with antidepressants, drawing a meaningful conclusion is premature.

Findings in previous studies in rats and nonhuman primates demonstrated that BDNF expression is regu-

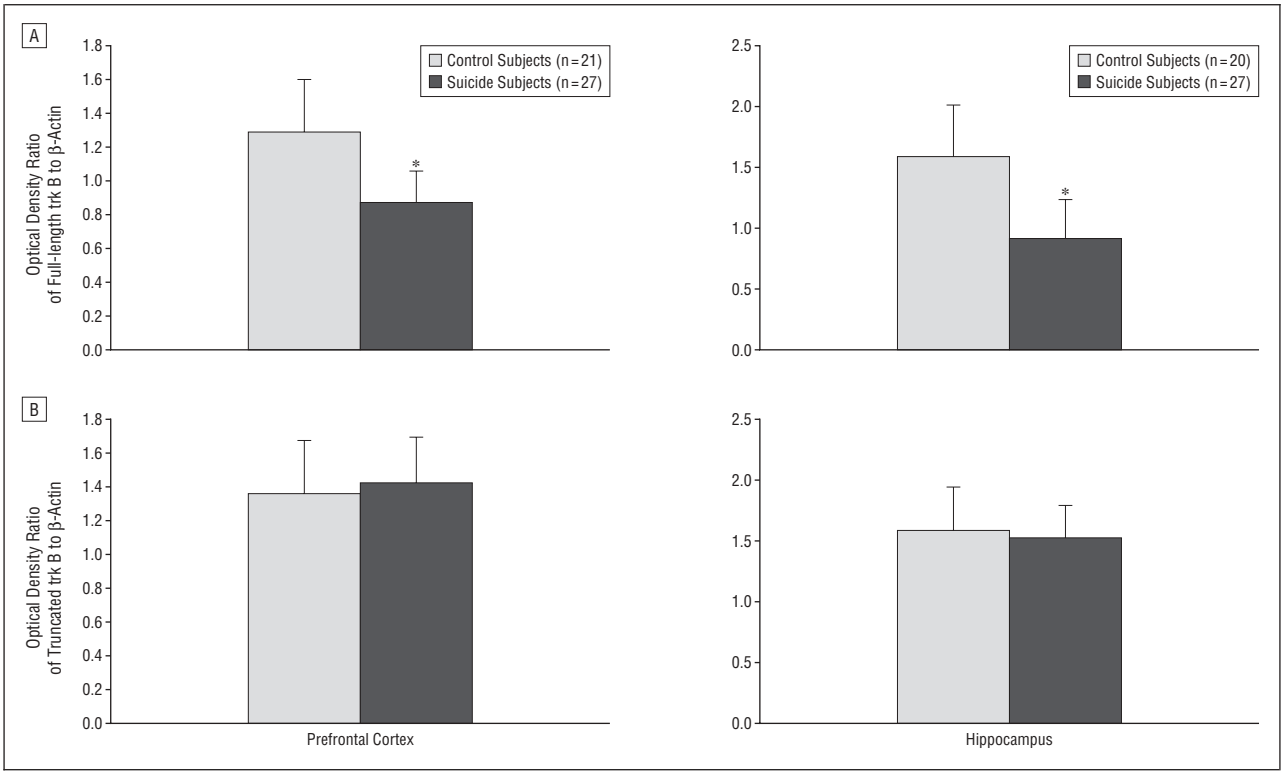

Figure 8. Immunolabeling of receptor tyrosine kinase B (trk B). Mean±SD of the optical density ratios of full-length (A) and truncated (B) trk B to β-actin in prefrontal cortex and hippocampus in control subjects and suicide subjects. Suicide group was compared with control group. The significance levels were as follows: for full-length trk B: cortex, t_{46}=7.53, P<.001 (95% confidence interval [CI], 0.26-0.58); hippocampus, t_{45}=7.65, P<.001 (95% CI, 0.45-0.92) and for truncated trk B: (cortex, t_{46}=0.75, P=.46 (95% CI, -0.24 to 0.10); hippocampus, t_{45}=0.62, P=.54 (95% CI, -0.13 to 0.25). Error bars indicate SD. Asterisks indicate P< .001. In 1 control subject, hippocampus was not available.

lated in response to stress. For example, immobilization stress, foot shock, and chronic unpredictable stress cause a rapid decrease in BDNF expression in the hippocampus and other limbic regions.[20-22] In addition, administration of antidepressants before the stress prevented the stress-induced decrease in hippocampal BDNF mRNA,[24] and systemic injection of glucocorticoids into rats lowered BDNF levels in the hippocampus and other brain areas.[38,39] The decreased expression of BDNF in suicide subjects could be related to stress. Although we have no direct evidence of stress abnormalities in the population in our study, results in other studies demonstrated a strong connection between stress-system overactivity and suicidal behavior.[3,4]

Another important observation of our study is the reduced expression of trk B in the prefrontal cortex and hippocampus in suicide subjects. BDNF binds specifically to trk B to mediate its biological functions. The trk B gene can give rise to at least 2 isoforms of trk B, encoding the full-length, or catalytic, form of trk B, the receptor mediating the main biological actions of BDNF,[30,31] and the truncated trk B receptors, which lack a large part of the intracellular domain and do not display protein-tyrosine kinase activity.[32] Binding with BDNF leads to activation of the full-length trk B receptors by ligand-induced dimer formation and autophosphorylation of tyrosine residues in the intracellular region.[40] The activated receptors become able to interact with and phosphorylate several intracellular targets. Although catalytic trk B is considered the receptor mediator of the main biological actions of BDNF, truncated trk B is also a predominant isoform in adult brain[41,42] and functions as cellular adhesion molecules regulating synaptic plasticity and axonal outgrowth, modulating signaling by catalytic trk B through the formation of heterodimers, and regulating the extracellular availability of its endogenous ligands.[32] BDNF signaling is impaired consequent to the formation of receptor heterodimers,[43] which suggests that truncated trk B can act as a negative modulator of BDNF signaling.

We found that only full-length trk B was lower in both prefrontal cortex and hippocampus in suicide subjects. This decrease may have serious implications, not only in terms of effects of BDNF-induced signaling but also regarding the supply of BDNF to neurons, and thus the loss of trophic maintenance of a variety of neuronal types because catalytic trk B is present predominantly within neuronal axons, cell soma, and dendrites.[44] In addition, the undiminished numbers of truncated trk B receptors will exacerbate any effects caused by the loss of catalytic trk B receptors, because truncated trk B receptors can inhibit BDNF-mediated neurite outgrowth by means of internalizing BDNF.

Authors of earlier preclinical and clinical studies demonstrated that the hippocampus is affected by stress. For example, neuronal atrophy and death were reported in the hippocampus in animals exposed to chronic stress or high levels of glucocorticoids.[9,12,20] Neuronal damage in the hippocampus has also been reported in monkeys subjected to social stress.[45] Humans with a history

Table 2. Messenger RNA and Protein Expression of BDNF and trk B in Prefrontal Cortex in Suicide Subjects With Major Depression and With Other Psychiatric Disorders*

Variable	Control Subjects (n = 21)	Suicide Subjects (n = 25)†		Overall Analysis of Variance			Multiple Comparison, *P* Value (95% CI)‡		
		With a History of Major Depression (n = 11)	With a History of Other Psychiatric Disorders (n = 14)	*df*	F	*P* Value	Controls vs Major Depression	Controls vs Other Psychiatric Disorder	Major Depression vs Other Psychiatric Disorder
BDNF mRNA§	606.8 ± 139.5	352.2 ± 133.9	367.0 ± 59.5	2, 43	24.6	<.001	<.001 (164.9 to 344.3)	<.001 (1598 to 326.1)	.81 (–108.7 to 85.4)
Ratio BDNF/NSE mRNA	1.75 ± 0.43	0.92 ± 0.37	1.03 ± 0.18	2, 43	25.7	<.001	<.001 (0.55 to 1.09)	<.001 (0.47 to 0.97)	.47 (–0.4 to 0.19)
BDNF protein‖	1.61 ± 0.39	0.94 ± 0.22	0.88 ± 0.28	2, 43	25.0	<.001	<.001 (0.41 to 0.92)	<.001 (0.49 to 0.95)	.67 (–0.21 to 0.32)
trk B mRNA§	1168.3 ± 322.3	739.4 ± 220.9	669.6 ± 230.5	2, 43	16.1	<.001	<.001 (220.6 to 637.4)	<.001 (299.6 to 686.0)	.57 (–161.7 to 289.5)
Ratio trk B/NSE mRNA	3.38 ± 1.02	1.94 ± 0.53	1.88 ± 0.66	2, 43	17.5	<.001	<.001 (0.82 to 2.07)	<.001 (0.89 to 2.06)	.93 (–0.65 to 0.71)
Full-length trk B protein‖	1.29 ± 0.31	0.83 ± 0.19	0.91 ± 0.19	2, 43	15.9	<.001	<.001 (0.27 to 0.66)	<.001 (0.22 to 0.58)	.52 (–0.27 to 0.14)
Truncated trk B protein‖	1.36 ± 0.32	1.46 ± 0.25	1.41 ± 0.29	2, 43	0.37	.42	0.40 (–0.32 to 0.13)	.52 (–0.27 to 0.14)	.82 (–0.22 to 0.27)

Abbreviations: BDNF, brain-derived neurotrophic factor; CI, confidence interval; mRNA, messenger RNA; NSE, neuron-specific enolase; trk B, receptor tyrosine kinase B.
*Data are given as mean ± SD unless otherwise indicated.
†In 2 suicide subjects, the diagnosis was not available.
‡The *P* values were compared with a Bonferroni-adjusted $\alpha = .05/5 = .01$; $\alpha = .01$ was considered significant.
§Expressed in attomoles per microgram of total RNA.
‖Expressed as the optical density ratio with β-actin.

Table 3. Messenger RNA and Protein Expression of BDNF and trk B in Hippocampus in Suicide Subjects With Major Depression and With Other Psychiatric Disorders*

Variable	Control Subjects (n = 20)†	Suicide Subjects (n = 25)‡		Overall Analysis of Variance			Multiple Comparison, *P* Value (95% CI)§		
		With a History of Major Depression (n = 11)	With a History of Other Psychiatric Disorders (n = 14)	*df*	F	*P* Value	Controls vs Major Depression	Controls vs Other Psychiatric Disorder	Major Depression vs Other Psychiatric Disorder
BDNF mRNA‖	2049.4 ± 642.8	1075.1 ± 200.6	1247.6 ± 372.4	2, 42	17.5	<.001	<.001 (600.1 to 1348.4)	<.001 (433.9 to 1128.5)	.34 (–594.6 to 208.5)
Ratio BDNF/NSE mRNA	5.02 ± 1.57	2.44 ± 0.45	3.13 ± 1.16	2, 42	17.1	<.001	<.001 (1.6 to 3.5)	<.001 (0.95 to 2.7)	.16 (–1.7 to 0.30)
BDNF protein¶	1.71 ± 0.44	1.04 ± 0.20	1.03 ± 0.22	2, 42	23.0	<.001	<.001 (0.41 to 0.91)	<.001 (0.44 to 0.90)	.97 (–0.26 to 0.27)
trk B mRNA‖	1325.4 ± 401.7	851.4 ± 355.4	746.3 ± 273.0	2, 42	12.3	<.001	.001 (203.0 to 744.9)	<.001 (319.1 to 822.2)	.51 (–194.2 to 387.5)
Ratio trk B/NSE mRNA	3.24 ± 0.99	1.94 ± 0.82	1.83 ± 0.63	2, 42	13.6	<.001	<.001 (0.65 to 1.95)	<.001 (0.78 to 1.99)	.79 (–0.61 to 0.79)
Full-length trk B protein¶	1.59 ± 0.42	0.84 ± 0.26	0.96 ± 0.36	2, 42	19.0	<.001	<.001 (0.47 to 1.04)	<.001 (0.36 to 0.88)	.37 (–0.44 to 0.17)
Truncated trk B protein¶	1.59 ± 0.36	1.47 ± 0.26	1.57 ± 0.28	2, 42	0.55	.58	.31 (–0.11 to 0.35)	.60 (–0.16 to 0.27)	.61 (–0.32 to 0.19)

Abbreviations: BDNF, brain-derived neurotrophic factor; CI, confidence interval; mRNA, messenger RNA; NSE, neuron-specific enolase; trk B, receptor tyrosine kinase B.
*Data are given as mean ± SD unless otherwise indicated.
†Hippocampus in 1 subject was not available.
‡In 2 suicide subjects, the diagnosis was not available.
§The *P* values were compared with a Bonferroni-adjusted $\alpha = .05/5 = .01$; $\alpha = .01$ was considered significant.
‖Expressed in attomoles per microgram of total RNA.
¶Expressed as the optical density ratio with β-actin.

of chronic recurrent depression or posttraumatic stress disorder exhibit substantial hippocampal atrophy in imaging studies.[46,47] Our findings that expression of BDNF and trk B is abnormal in both prefrontal cortex and hippocampus clearly indicate that both brain areas could be equally important in suicidal behavior. This finding

is supported by recent observations that prefrontal cortical areas show structural abnormalities, including low neuronal density and loss of glial cells, in patients with affective disorder.[6,7] That both trk B and BDNF are present in neurons and in glia[48] does not necessarily imply glial synthesis of BDNF but may alternatively indicate BDNF internalization or the presence of receptor-bound BDNF. In the future, it will be important to examine whether the expression of BDNF is lower in neuronal and/or glial cells in those who commit suicide.

The mechanism by which BDNF expression is decreased in the prefrontal cortex and hippocampus in suicide subjects is not yet clear. Mature BDNF transcripts are formed from the 5 exons (1-5) that make up the BDNF gene by splicing 1 of the first 4 exons to the fifth.[49] Recent results suggest that Ca^2+ influx couples to the pathways that lead to the activation of CREB and that CREB activation increases BDNF transcription through Ca^2+/cyclic adenosine monophosphate response element within exon 3 of BDNF.[50] CREB is activated not only through the Ca^2+-induced signaling pathway but also through protein kinase A, an important component of the adenylyl cyclase pathway. In recent studies, we found that the catalytic activity of protein kinase A[51] and the expression and functional characteristics of CREB[29] are lower in postmortem brain in suicide subjects. Decreases in protein kinase A and CREB could be responsible for the decreased expression of BDNF. Other possible regulators of BDNF expression could be neurotransmitters and hormones. For example, Lauterborn et al[52] showed that the expression of BDNF exons 1 and 2 is negatively modulated by adrenal steroids. Serotonin also regulates the expression of the BDNF gene.[24,53] Recently, Vaidya et al[22] have demonstrated that pretreatment with a selective $5HT_{2A}$ receptor antagonist blocks stress-induced decrease in BDNF expression. Interestingly, both serotonin[54-56] and adrenal steroids[57-59] have been implicated in suicidal behavior.

The pathophysiological importance of abnormal expression of BDNF and trk B in postmortem brain in suicide subjects remains to be elucidated; however, given the importance of these proteins in cellular proliferation and cell survival during adulthood, a decrease in BDNF and trk B would lead to atrophy or loss of neurons and/or glial cells. The reported structural abnormalities in the brain in patients with affective disorder, and in the general population during stress, could be associated with decreases in BDNF and trk B. In addition, BDNF participates in neural plasticity; findings of several studies demonstrated the direct link of neurotrophins to long-term changes in synaptic strength, effects that may contribute to learning and memory.[60,61] Mesulam[62] discussed the possibility that failure of the mechanisms underlying neural plasticity could contribute to neurodegenerative disorders. More recently, Duman et al[63] proposed that stress-related affective illness may result in part from a loss of neuronal plasticity, the process mediated by the stress hormone by which the brain adapts at the cellular and molecular levels to changes in its environment. This hypothesis could also be relevant in suicidal behavior, in which affective illness and stress are the major contributory factors. Another important function of BDNF is the regulation of growth and functions of 5HT-containing neurons in the adult brain. Long-term infusion of BDNF into rat midbrain increases 5HT turnover in many brain areas,[64,65] and in the neocortex it causes sprouting of 5HT nerve terminals and accelerates the regrowth of serotonergic nerve fibers after destruction by parachloroamphetamine.[66] Diminished BDNF thus may contribute to reduced 5HT turnover and decreased trophic support of 5HT-containing neurons, and results of several studies, including our own, demonstrate that the serotonergic system plays an important role in depression and suicidal behavior.[54-56]

Thus, given the importance of BDNF and trk B in various biological actions in the brain, our findings of decreased expression of these molecules in postmortem brain in suicide subjects are of critical importance and suggest the possibility that both of these molecules may serve as important vulnerability factors in predisposing a person to suicidal behavior. Furthermore, results of this study provide a possible molecular basis for the structural brain impairments reported in patients with affective disorders, during stress, and in suicide subjects.

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