Brain-derived neurotrophic factor and tyrosine kinase B receptor signalling in post-mortem brain of teenage suicide victims

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Abstract
Teenage suicide is a major public health concern, but its neurobiology is not very well understood. Stress and major mental disorders are major risk factors for suicidal behaviour, and it has been shown that brain-derived neurotrophic factor (BDNF) and its receptor tyrosine kinase B (TrkB) are not only regulated by stress but are also altered in these illnesses. We therefore examined if BDNF/TrkB signalling is altered in the post-mortem brain of teenage suicide victims. Protein and mRNA expression of BDNF and of TrkB receptors were determined in the prefrontal cortex (PFC), Brodmann’s Area 9 (BA 9), and hippocampus obtained from 29 teenage suicide victims and 25 matched normal control subjects. Protein expression was determined using the Western blot technique; mRNA levels by a quantitative RT–PCR technique. The protein expression of BDNF was significantly decreased in the PFC of teenage suicide victims compared with normal control subjects, whereas no change was observed in the hippocampus. Protein expression of TrkB full-length receptors was significantly decreased in both PFC and hippocampus of teenage suicide victims without any significant changes in the truncated form of TrkB receptors. mRNA expression of both BDNF and TrkB was significantly decreased in the PFC and hippocampus of teenage suicide victims compared with normal control subjects. These studies indicate a down-regulation of both BDNF and its receptor TrkB in the PFC and hippocampus of teenage suicide victims, which suggests that stress and altered BDNF may represent a major vulnerability factor in teenage suicidal behaviour.

Introduction
Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin (NT) family of highly basic proteins, which includes nerve growth factor (NGF), NT-3, NT-4, and NT-5 besides BDNF (Huang and Reichardt, 2001). These growth factors are involved in cellular proliferation and migration, differentiation, and survival of neurons in the human central nervous system (CNS). They are also involved in neuronal regeneration and the maintenance of neuroplasticity in adults, as they also regulate synaptic activity and neurotransmitter synthesis (Altar et al., 1997; Bartrup et al., 1997; Huang and Reichardt, 2001; Kang and Schuman, 1995; Schinder and Poo, 2000; Thoenen, 1995). The specific functions of each neurotrophin are not very well understood, but animal knockout studies of neurotrophins or their receptors indicate that each family member may support some specific neuronal subpopulation. Of the neurotrophins, BDNF and its role in affective illness and schizophrenia has been the most widely studied (Buckley et al., 2007a,b; Cunha et al., 2006; Dwivedi et al., 2003b; Fukumoto et al., 2001; Hashimoto et al., 2002; Machado-Vieira et al., 2007; Shimizu et al., 2003; Shoval and Weizman, 2005; Toyooka et al., 2002), but its role in teenage suicide has not been examined.

It is believed that BDNF produces its physiological effects by binding with its receptor, known as the tyrosine kinase B (TrkB) receptor (Barbacid, 1994; Huang and Reichardt, 2001). Other neurotrophins,
such as NGF and NT-3, bind to TrkA and TrkC receptors respectively (Dechant et al., 1994). Binding of BDNF to the TrkB receptor initiates a signalling cascade that is thought to be mediated through the Ras/mitogen-activated protein (MAP) kinase, phosphoinositide (PI) 3-kinase (Mizuno et al., 2003), and phospholipase C (PLC) pathways. Trk receptors are single-pass transmembrane proteins that are highly expressed in developing neural systems. They dimerize in response to their activation by neurotrophins, and the dimeric receptors phosphorylate one another. Rapid autophosphorylation of the tyrosines within the Trk receptors’ activation loop further initiates the catalytic activity of the kinase, e.g. further phosphorylation of PLC by TrkB activates this enzyme and initiates the PI signalling cascade (Segal, 2003).

BDNF is regulated by stress, mental illness, and serotonergic mechanisms, which are all major risk factors for suicidal behaviour (Malone et al., 1995; Paykel, 1976; Westrin, 2000). For example, it has been shown that immobilization stress causes down-regulation of BDNF mRNA in the rat hippocampus (Smith et al., 1995; Ueyama et al., 1997). Vaidya et al. (1999) examined the role of 5-HT2A receptors in stress-induced down-regulation of BDNF and found that pre-treatment with ketanserin significantly blocked the stress-induced decrease of BDNF mRNA in animals subjected to immobilization stress. These results suggest that not only stress but also serotonergic mechanisms are involved in the regulation of BDNF.

BDNF has also been shown to be a target for the action of antidepressant drugs (Chen et al., 2001). Treatment with antidepressants or electroconvulsive shock (ECS) causes an increase in BDNF levels in the rat brain (Altar et al., 2003; Nibuya et al., 1995). More recently, some investigators have shown that levels of BDNF are decreased in depressed suicide victims (Dwivedi et al., 2003b; Karege et al., 2005b) but are increased in the post-mortem brain of antidepressant-treated depressed suicide victims (Chen et al., 2001). Furthermore, levels of BDNF have been found to be decreased in the serum of depressed (Karege et al., 2002, 2005a; Shimizu et al., 2003), bipolar (Cunha et al., 2006; Machado-Vieira et al., 2007), and schizophrenia patients (Buckley et al., 2007a,b; Shoval and Weizman, 2005). Structural abnormalities, as well as reduction in neuronal density and size, have been reported in the post-mortem brain of depressed suicide victims (Rajkowska, 1997, 2000). Several studies do suggest that mood disorders may be associated with abnormalities of neuroplasticity and neuronal atrophy. Since mental disorders are major risk factors for suicide, and since BDNF is involved in regulating neuronal development and survival, this suggests the involvement of BDNF in the pathophysiology of suicide. Taken together, these observations do suggest an important role of BDNF signalling in the pathophysiology of suicidal behaviour.

Suicide is a major public health concern. Each year about 30 000 people die by suicide in the USA alone (Botsis et al., 1997; Moscicki et al., 1988). In teenagers, it is the second largest cause of death; hence, it is an important national public health concern. Although there is some understanding of the neurobiology of adult suicidal behaviour, very little is known about the neurobiology of teenage suicide. We previously reported a decrease in the protein and mRNA expression of BDNF and TrkB in the prefrontal cortex (PFC) and hippocampus of adult depressed suicide victims (Dwivedi et al., 2003b, 2005). This has not been studied in teenage suicide victims, whose characteristics may be slightly different from those of adult suicide victims. To examine the role of BDNF signalling in teenage suicide we have determined the protein and mRNA expression of BDNF and its receptor TrkB in the PFC and hippocampus obtained from teenage suicide victims and matched normal control subjects.

Methods and materials

Acquisition of human post-mortem brain samples

Brain tissues were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, Maryland, in collaboration with the Office of the Chief Medical Examiner of the State of Maryland. Tissue samples were obtained from 29 teenage suicide victims and from 25 teenage control subjects (Table 1). Toxicological data were obtained by analysis of urine and blood samples from these subjects. All procedures were approved by the University of Maryland Institutional Review Board.

All subjects in this study were diagnosed using the Diagnostic Evaluation After Death (DEAD; Salzman, 1983) and the Schedule for Clinical Interviews for DSM-IV (SCID; First et al., 1997). The SCID was administered by a trained interviewer using a family member as an informant, as well as from a review of all obtainable medical and psychiatric records. DEAD is used only as a data organization instrument. SCID diagnoses are validated by two trained psychiatrists. This has been found to be a very accurate way to make diagnoses (see Ramirez Basco et al., 2000). Family members gave permission for the use of brain tissue for research and for clinical records.
to be obtained from mental health treatment providers when there was a prior history of mental health treatment, or suicide. Two senior psychiatrists provided independent DSM-IV diagnoses. Similarly, normal controls were verified as free from mental illnesses using such consensus diagnostic procedures.

Immunolabelling of BDNF and TrkB

The procedure for Western blotting has been described in detail (Dwivedi et al., 2003b). PFC or hippocampus was homogenized (1:6, w/v; i.e. 100 mg of tissue were homogenized in 0.6 ml buffer) in a buffer containing 100 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium decanoate, 0.1% SDS, 5 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml aprotinin, leupeptin, pepstatin, and 100 mM sodium orthovanadate. The homogenate was centrifuged at 15 000 g for 10 min at 4°C and the supernatant was used for all the assays. Protein concentration was determined according to the method of Lowry et al. (1951). Protein samples (30 μg) were loaded onto 15% (w/v) sodium dodecyl sulphate (SDS)–polyacrylamide gel for BDNF and 10% (w/v) for TrkB. The gels were run and transferred electrophoretically to an enhanced chemiluminescence (ECL) nitrocellulose membrane (Amersham, Arlington Heights, IL, USA). The membranes were washed with TBST buffer [10 mM Tris-base, 0.15 M NaCl, and 0.05% (v/v) Tween-20] for 10 min. The blots were blocked by incubating with 5% (w/v) powdered non-fat milk in TBST, 0.02% Nonidet P-40, and 0.02% (w/v) SDS (pH 8.0). Then the blots were incubated overnight at 4°C with primary polyclonal anti-TrkB antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a dilution of 1:3000 and primary monoclonal anti-human BDNF antibody (R & D Systems Inc., Minneapolis, MN, USA) at a dilution of 1:500. The membranes were washed with TBST and incubated with horseradish-peroxidase-linked secondary antibody [anti-rabbit immunoglobulin G (IgG); 1:2000] for 5 h at room temperature. The membranes were extensively washed with TBST and exposed to ECL autoradiography film. The same nitrocellulose membrane was stripped and re-probed with β-actin antibody (Sigma Chemical Co., St Louis, MO, USA). The bands on the autoradiogram were quantified using the Loats Image Analysis System (Loats Associates Inc., Westminster, MD, USA), and the optical density of each sample was corrected by the optical density of the corresponding β-actin band. The values are represented as a percent of the control.

Quantitative reverse transcriptase–polymerase chain reaction (RT–PCR) analysis of BDNF, TrkB, and cyclophilin A (CyP) mRNA levels

Total RNA was extracted from ~100 mg of tissue using TRIzol® reagent (Invitrogen Corp., Carlsbad, CA, USA). RNA was quantified by NanoDrop® (Thermo Fisher Scientific, Waltham, MA, USA), and quality of total RNA was determined by the visual assessment of the 28S:18S rRNA ratio on a denaturing agarose gel. In addition, to determine the integrity of RNA, all samples were diluted to a concentration of 100 ng/μl and a 1 μl aliquot was analysed using the lab-on-a-chip technology of the Agilent Bioanalyzer 2100 (Agilent Technology, Palo Alto, CA, USA). RNA Integrity Numbers (RINs) were generated using Agilent 2100 Expert software as a criterion of the RNA quality for downstream experiments. The RIN values are scaled from number 1 (RNA completely degraded) to 10 (intact RNA). To exclude the possibility of using degraded RNA, only samples with an RNA integrity number > 5 were used.

Quantitation of BDNF, TrkB, and CyP mRNA was performed using internal standards. CyP was used as a housekeeping gene. Cloning and synthesis of internal standards were done as described previously (Dwivedi et al., 2002). Primer pairs, for each gene, were designed to allow amplification of 253–567 bp for BDNF (GenBank accession no. X91251); forward: 5'-AAGGACCGCAGACTTGTACACG (252–273 bp); reverse: 3'-CATGGGATTGCACCTTGCTC (547–567 bp); 599–969 bp for TrkB (GenBank accession no. S76473); forward: 5'-ATCTGGCCGCACCTAA-CCTCA (599–619 bp); reverse: 3'-TATTGCCCGGCT-TATAGAACCA (949–969 bp); and 118–421 bp for CyP (GenBank accession no. XM_371409); forward: 5'-AGC ACT GGA GAG AAA GGA TTT G (118–139 bp); reverse, 5'-CCT CCA CAA TAT TCA TGC CCT T (400–421 bp). Internal primer sequences: BDNF (5'-CGAGGAGTTCTGGAGCTGTTG); TrkB (AACGCCATCTGGAGCTGTTG); and cyclophilin A (5'-CATGCTGAGTGGCCGGA). The underlined sequences indicate the BglII site for BDNF and the XhoI site for TrkB, whereas the bold and italicized letters indicate the mutated bases. The internal standard for CyP was generated by deleting a 65-bp fragment (220–237/303–320 bp): 5'-GTT GGC AAG TCC ATC TAT/AAA TGC TGG ACC CAA CAC between the amplification primers. Each of the internal standards was synthesized in two PCR steps, starting with a cDNA template reverse transcribed from the total RNA. The internal standard templates were first cloned into a pGEM4Z vector and then amplified using M13 primers. The cRNA corresponding to the
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sense strand was synthesized from an M13 amplified template using an Sp6 RNA polymerase MAXIscript Kit (Ambion Inc., Austin, TX, USA). All subcloned target genes were sequenced using M13 primers to make sure they matched the corresponding sequences reported in GenBank.

The quantitation of BDNF, TrkB, and CyP mRNA was accomplished by the competitive RT–PCR method using internal standards as described earlier (Dwivedi et al., 2003b). Decreasing concentrations of internal standard cRNA, BDNF (5–1.56 pg), TrkB (400–12.5 pg), or CyP (200–12.5 pg), were added to 1 μg total RNA, and the RNA/cRNA mixtures were denatured at 80 °C for 6 min and then reverse transcribed with cloned M-MLV reverse transcriptase in RT buffer containing 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂, 1 mM dNTPs using random hexamers (2.5 μM) and ribonuclease inhibitor (28 U) in a volume of 20 μl. The RT mixture was incubated at 37 °C for 60 min to promote cDNA synthesis. The reaction was terminated by heating the samples at 98 °C for 5 min. In all assays, one RT reaction was performed in the absence of RNA as a control. Competitive PCR amplification of cDNA aliquots containing reverse transcribed material was performed with Hot Tub DNA polymerase (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). The amplification mixture contained cDNA, 0.5 μM specific primer pairs, 200 μM dNTPs, 1.5 mM MgCl₂, 50 mM Tris–HCl (pH 9.0), 20 mM ammonium sulphate, 15 mM KCl, and 1.5 U of Hot Tub DNA polymerase in a 50 μl volume. Trace amounts of [³²P]dCTP (0.5 μCi) were included during the PCR step for subsequent quantification. The PCR mixture was amplified for 30 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). Following amplification, aliquots were
digested with BglII or XhoI and run on a 1.5% agarose gel. To quantitate the amount of product corresponding to the reverse-transcribed and amplified mRNA, the ethidium bromide-stained bands were excised and counted. The results were calculated as the counts incorporated into the amplified cRNA standard divided by the counts incorporated into the corresponding mRNA amplification product vs. the known amount of internal standard (cRNA) added to the test sample. The results are expressed as attomoles/μg of total RNA.

Statistical analysis

Statistical differences in age, post-mortem interval (PMI), gender, and the protein and mRNA expression of BDNF and TrkB between normal controls and suicide victims were evaluated by Student’s t test. The relationships between age, gender, PMI, brain pH, race and the protein and mRNA expression of BDNF and TrkB were determined by Pearson’s product-moment correlation analysis. Differences between suicide victims with and without mental disorders were evaluated by one-way ANOVA.

Results

The demographic details and characteristics of the teenage suicide victims (n =29) and control subjects (n = 25) are given in Table 1. The age range of the teenage suicide victims was from 13 to 20 yr. There were no significant differences in the mean age (t = 0.4, d.f. = 52, p = 0.69) or in the mean PMI (t = 0.46, d.f. = 51, p = 0.65) between the suicide victims and the normal control subjects. We determined brain pH in the samples by homogenizing a small piece of cerebellum in distilled water and measuring the acidity with a pH meter. There were no significant differences in the mean pH values between suicide victims and normal control subjects (t = 0.19, d.f. = 49, p = 0.84).

Protein expression of BDNF in the PFC and hippocampus of suicide victims and normal control subjects

We determined the protein expression of BDNF using a specific monoclonal antibody, and as expected, the antibody detected a 14 kDa band in the cellular extract. Representative Western blots showing the protein levels of BDNF and β-actin in the PFC of six suicide victims and six control subjects are shown in Figure 1a. The protein expression levels were corrected by the levels of β-actin in each case. β-Actin was used as a housekeeping protein, and we did not find any significant differences in the levels of β-actin in either brain area between suicide victims and normal control subjects. Therefore ratios of BDNF to β-actin were calculated to determine the protein expression levels of BDNF. The mean protein expression levels of BDNF in the PFC and hippocampus of teenage suicide victims (n =29) and control subjects (n = 25). Values are the mean ± S.D. TC, Teenage controls; TS, teenage suicide victims (** p <0.001).

Immunolabelling of full-length and truncated TrkB receptors

As stated earlier, the TrkB receptors exist as at least two isoforms, the full-length, or catalytic form, and the truncated TrkB receptors, which lack a large part of the intracellular domain and do not display protein kinase activity. We therefore determined the immunolabelling of both types of TrkB receptors separately using specific antibodies in both PFC and hippocampus of teenage suicide victims and normal control subjects. Representative Western blots of both full-length and truncated TrkB receptors in the PFC of six suicide victims and six control subjects are shown.
in Figure 2a. The full-length TrkB receptor migrated to 145 kDa, whereas the truncated TrkB migrated to 95 kDa. Ratios of full-length or truncated TrkB vs. β-actin, the housekeeping protein, were calculated for comparison analysis. When we compared the expression of TrkB receptors, we found that the protein expression of the full-length TrkB receptors was significantly decreased in the PFC obtained from teenage suicide victims, whereas there was no significant difference in the protein expression of the truncated TrkB receptors between teenage suicide victims and normal control subjects (Figure 2b). The protein expression of the full-length TrkB receptors in the hippocampus was also found to be significantly decreased in suicide victims compared with normal control subjects, without any significant difference in the truncated TrkB receptors between suicide victims and normal control subjects (Figure 2b).

mRNA expression levels of BDNF in the PFC and hippocampus of teenage suicide victims

Initially we determined the RIN for all the samples to examine the quality of the RNA. We found that the mean RIN was 5.86 ± 0.61 for normal controls and 5.90 ± 0.45 for teenage suicide victims. These are acceptable RINs for mRNA expression studies. We next examined if the changes in the protein expression levels of BDNF observed in the PFC of suicide victims are associated with the changes in mRNA levels. Using the competitive RT–PCR technique, we determined BDNF mRNA levels in the PFC and hippocampus of suicide victims and normal control subjects. A representative gel electrophoresis of the competitive RT–PCR of BDNF in the PFC from one control subject is given in Figure 3a, and the competitive RT–PCR

Figure 2. (a) Representative Western blots showing the immunolabelling of full-length and truncated tyrosine kinase B (TrkB) and β-actin in the prefrontal cortex (PFC) from six control subjects and six teenage suicide victims. (b) Mean protein levels of full-length or truncated TrkB in the PFC and hippocampus of teenage suicide victims (n = 29) and control subjects (n = 25). Values are the mean ± S.D. TC, Teenage controls; TS, teenage suicide victims (* p < 0.01, ** p < 0.001).

Figure 3. Representative agarose gel electrophoresis showing a competitive RT–PCR for (a) brain-derived neurotrophic factor (BDNF) mRNA and (b) BDNF mRNA content in prefrontal cortex (PFC) obtained from one control subject. 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. (c) The mean ± S.D. of BDNF mRNA in PFC and hippocampus in control subjects and suicide victims. Suicide group was compared with control group (error bars indicate S.D.). TC, Teenage controls; TS, teenage suicide victims (** p < 0.001).
analysis is presented in Figure 3b, where the point of equivalence represents the amount of BDNF present. The mean mRNA expression levels of BDNF in the PFC and hippocampus of teenage suicide victims and normal controls are shown in Figure 3c. We found that the mRNA levels of BDNF were significantly decreased in both PFC and hippocampus of suicide victims compared with normal control subjects (Figure 3c).

We then determined the mRNA levels of TrkB receptors in the PFC and hippocampus of teenage suicide victims and normal controls. The mean mRNA expression levels of TrkB receptors in PFC and hippocampus of teenage suicide victims and normal control subjects are shown in Figure 4c, and as can be seen, there was a significant decrease in the mRNA levels of TrkB receptors in the PFC, as well as hippocampus, of teenage suicide victims compared with normal control subjects.

To normalize the data, we used CyP as a housekeeping gene. We first determined the mRNA levels of CyP in both PFC and hippocampus of suicide victims and control subjects, and then the ratios of BDNF or TrkB mRNA to that of CyP mRNA in the PFC and hippocampus of suicide victims and control subjects. The results are shown in Figure 5a, b respectively. We observed that the ratios of mRNA levels between BDNF and CyP, as well as of TrkB.
and CyP, were still significantly lower in teenage suicide victims compared with normal control subjects. These results thus suggest that the observed decreases in the mRNA levels of BDNF and TrkB receptors in the teenage suicide victims, compared with normal control subjects, were not a function of non-specific mRNA loss as a result of neuronal damage.

Effects of diagnosis on the protein and mRNA expression levels of BDNF and of TrkB receptors

Since alterations in BDNF and TrkB have been suggested to be involved in the pathophysiology of depression and bipolar (BP) illness, it was of interest to examine if the changes in BDNF or TrkB are related to a specific diagnosis. Of the 29 suicide subjects, seven had a primary diagnosis of major depressive disorder (MDD), six had conduct or adjustment disorders, two had schizophrenia, two had alcohol abuse, one had attention deficit hyperactivity disorder, one had borderline personality disorder, one had dissociative disorder and nine had no documented history of mental disorders but had some mental disorder with conduct or adjustment disorder. We compared BDNF and TrkB protein and mRNA expression among the subjects with MDD \((n=7)\) and conduct or adjustment disorders \((n=6)\) and with no documented mental illness \((n=9)\). There was no significant difference between any of these groups in the protein or mRNA expression of BDNF or TrkB in either PFC or hippocampus. These analyses suggest that the changes in BDNF and TrkB we observed are not related to a particular diagnosis but may be specific to suicide.

Effects of confounding variables

To rule out the possibility that the observed changes in mRNA and protein levels of BDNF and TrkB in the PFC of the suicide victims are related to possible confounding variables, we examined the effects of age, gender, PMI, brain pH, and race on these measures. There was no significant correlation of age, PMI, gender, or brain pH with any of the measures studied. For example, the potential confounding variables had no significant effect on protein levels of BDNF (PFC: age, \(r=0.1, p=0.48\); PMI, \(r=0.07, p=0.59\); pH, \(r=0.17, p=0.24\). Hippocampus: age, \(r=0.13, p=0.36\); PMI, \(r=0.02, p=0.87\); pH, \(r=0.06, p=0.69\)) or of full-length TrkB (PFC: age, \(r=0.23, p=0.11\); PMI, \(r=0.08, p=0.59\); pH, \(r=0.22, p=0.13\). Hippocampus: age, \(r=0.04, p=0.77\); PMI, \(r=0.09, p=0.49\); pH, \(r=0.008, p=0.96\)). Similarly, age, PMI, or pH had no effect on mRNA levels of BDNF (PFC: age, \(r=0.19, p=0.16\); PMI, \(r=0.26, p=0.06\); pH, \(r=0.14, p=0.34\). Hippocampus: age, \(r=0.006, p=0.97\); PMI, \(r=0.06, p=0.66\); pH, \(r=0.19, p=0.19\) or of TrkB (PFC: age, \(r=0.08, p=0.57\); PMI, \(r=0.19, p=0.16\); pH, \(r=0.03, p=0.84\). Hippocampus: age, \(r=0.02, p=0.89\); PMI, \(r=0.12, p=0.43\); pH, \(r=0.22, p=0.13\)). We also examined whether race had any effect on BDNF or TrkB. There were 31 white and 21 black subjects. We did not find any significant effect of race either on the protein levels of BDNF (PFC: \(r=0.01, p=0.95\); hippocampus: \(r=0.005, p=0.98\)) or of TrkB (PFC: \(r=0.113, p=0.60\); hippocampus: \(r=0.10, p=0.64\)) or on the mRNA levels of BDNF (PFC: \(r=0.09, p=0.64\); hippocampus: \(r=0.33, p=0.11\)) or of TrkB (PFC: \(r=0.04, p=0.84\); hippocampus: \(r=0.15, p=0.46\)). We also found no significant differences in protein (PFC: \(t=1.45, d.f. =22, p=0.16\); hippocampus: \(t=0.12, d.f. =23, p=0.91\)) and mRNA (PFC: \(t=0.67, d.f. =23, p=0.51\); hippocampus: \(t=0.64, d.f. =23, p=0.53\)) levels of BDNF, or protein (PFC: \(t=0.14, d.f. =21, p=0.89\); hippocampus: \(t=1.46, d.f. =22, p=0.08\)) or mRNA (PFC: \(t=0.93, d.f. =23, p=0.36\); hippocampus: \(t=0.46, d.f. =23, p=0.65\)) levels of TrkB between male and female subjects in the normal control group.

Since it has been shown that antidepressant treatment causes an increase in BDNF (Altar et al., 2003; Nibuya et al., 1995), we therefore also compared the protein and mRNA expression levels of BDNF and TrkB in the four teenage suicide victims who had a history of antidepressant treatment and who had antidepressants present in their blood at the time of death with those of suicide subjects with no antidepressant treatment. Their BDNF and TrkB protein and mRNA expression levels were not significantly different. We also compared the suicide victims who had no antidepressant treatment \((n=25)\) with normal controls, and the results were similar to those obtained when we included the suicide victims with antidepressant treatment.

Discussion

In this study we examined the role of BDNF and its receptor TrkB in the pathophysiology of teenage suicide by determining the protein and mRNA levels in two areas of human post-mortem brain, i.e. PFC (BA 9) and hippocampus, from teenage suicide victims and matched normal control subjects. Since BDNF signalling occurs by means of the binding of BDNF to its receptor TrkB (Segal, 2003), we also determined the protein expression levels of the full-length and the truncated forms of TrkB receptors. The important findings of this study are that (1) the
protein and mRNA levels of BDNF are significantly decreased in the PFC of teenage suicide victims compared with normal controls; (2) in the hippocampus there is no significant change in the protein expression levels of BDNF but the BDNF mRNA levels are significantly decreased in teenage suicide victims compared with normal controls; and (3) the mRNA expression of TrkB and the protein levels of the TrkB full-length receptor are significantly decreased in both PFC and hippocampus of teenage suicide victims compared with normal controls; however, there are no significant changes in the protein levels of the truncated TrkB receptors in the PFC or hippocampus of teenage suicide victims compared with normal control subjects.

There is both direct and indirect evidence suggesting the involvement of BDNF in suicidal behaviour (Chen et al., 2001; Dwivedi et al., 2003b; Karege et al., 2005b), mood disorders and schizophrenia (Buckley et al., 2007a,b; Cunha et al., 2006; Dwivedi et al., 2003b; Fukumoto et al., 2001; Hashimoto et al., 2002; Lang et al., 2004; Machado-Vieira et al., 2007; Shimizu et al., 2003; Shoval and Weizman, 2005; Toyooka et al., 2002). Mood disorders and schizophrenia are major risk factors for suicidal behaviour, and the evidence that BDNF may play a role in mental disorders may also implicate its involvement in suicidal behaviour. The evidence that BDNF is involved in mood disorders is derived from the observation that long-term treatment with antidepressant and mood-stabilizing drugs, especially selective serotonin reuptake inhibitors (SSRIs), ECS, or lithium, causes the up-regulation of BDNF mRNA in rat brain (Altar et al., 2003; Duman and Vaidya, 1998; Fukumoto et al., 2001; Hashimoto et al., 2002; Nibuya et al., 1995). Similarly BDNF level has also been reported to be increased after treatment with monoamine oxidase (MAO) inhibitors in rat brain (Altar et al., 2003).

More direct evidence for the involvement of BDNF in depression has been provided by the studies of BDNF in the serum and/or post-mortem brains obtained from depressed and/or suicide subjects. For example, Karege et al. (2002) and others (Chen et al., 2001; Cunha et al., 2006; Dwivedi et al., 2003b; Machado-Vieira et al., 2007; Shimizu et al., 2003) found a decreased level of serum BDNF in bipolar or depressed patients. Chen et al. (2001) observed an increased level of BDNF immunoreactivity in the post-mortem brain of those depressed patients who were treated with antidepressants. Since treatment with antidepressants can cause an increase in BDNF levels, it was suggested that this increase is probably related to the antidepressant treatment. Direct evidence suggesting a decrease in BDNF levels in depressed suicide victims was recently provided by Dwivedi et al. (2003b), who reported decreased protein and mRNA expression levels of BDNF in the PFC and hippocampus of adult suicide victims. Karege et al. (2005b) also found decreased protein expression of BDNF in the PFC and hippocampus of suicide victims. Decreased BDNF levels have also been reported in the serum/plasma of schizophrenia patients (Buckley et al., 2007a,b; Shoval and Weizman, 2005; Toyooka et al., 2002).

An important question is: What is the mechanism that causes the down-regulation of BDNF in the post-mortem brain of teenage suicide victims? One possible mechanism may involve alterations in serotonergic mechanisms. Some studies suggest that levels of BDNF may be regulated by serotonergic mechanisms (Nibuya et al., 1995; Zetterstrom et al., 1999). Treatment with SSRIs used as antidepressant drugs causes the up-regulation of BDNF mRNA in rat brain (Nibuya et al., 1995). Since some of these antidepressants may produce their effects through their actions on 5-HT$_{2A}$ receptor subtypes, Vaidya et al. (1999) examined if antidepressants exerted such effects on BDNF mRNA through the 5-HT receptor subtypes. They found that treatment with the 5-HT$_{2A}$ agonist 4-iodo-2,5-dimethoxyphenylisopropylamine (DOI) increased BDNF mRNA in cortex and hippocampus of rats whereas treatment with a 5-HT$_{2A}$ antagonist, ketanserin or R-(-)-alpha-(2,3-dimethoxyphenyl)-1-[2(4-fluorophenylethyl)]-4-piperidine-methanol (MDL 100,907), blocked the effects of DOI in increasing the 5-HT$_{2A}$ receptor-mediated increase in BDNF mRNA. This effect does not, however, appear to be related to the 5-HT$_{2A}$ receptors. Evidence that the decrease in BDNF in the post-mortem brain of teenage suicide victims may in fact be related to changes in 5-HT receptors is provided by our findings that the protein and mRNA expression of 5-HT$_{2A}$ receptors is increased in the post-mortem brain of teenage suicide victims, both in the PFC as well as in the hippocampus (Pandey et al., 2002). Consequently, it is also possible that the altered level of BDNF may be regulating these serotonergic activities. It has been reported by Altar et al. (1994) that a midbrain infusion of BDNF causes a significant increase in the levels of 5-hydroxyindole acetic acid (5-HIAA) and in the 5-HIAA:5-HT ratio in all areas of rat brain, including hippocampus and cortex. These observations suggest that BDNF may increase the synthesis and/or release of 5-HT, and that the decreased serotonergic activity and decreased CSF 5-HIAA observed in suicidal
patients may be related to decreased levels of BDNF. Such may also be the consequences of decreased BDNF in the suicide brain.

**BDNF signalling in suicide: role of TrkB receptors**

Recent studies have suggested that several important pathways are activated by BDNF. The first step in the activation of signalling pathways is the binding of a neurotrophin to its specific Trk receptor through which each neurotrophin produces its physiological effects. BDNF binds to the TrkB receptors, which exist in two forms, the full-length catalytic form and the truncated form, which lacks the tyrosine kinase domain and thus is devoid of tyrosine kinase activity (Middlemas et al., 1991). In the brain, the catalytic TrkB receptor is expressed in the neuron and appears to be neuron specific. Although initial studies suggested that the truncated form may not be present in neurons, since it was formed in the choroid plexus and ependymal cell layer of certain ventricles (Klein et al., 1991), more recent work (Armanini et al., 1995) has suggested that the truncated form is expressed in both neurons as well as glia (Lindsay et al., 1994). The catalytic form mediates the main biological functions of BDNF, as well as retrograde transport of BDNF to neuronal cells (Altar et al., 1997). The function of the truncated TrkB receptor is unknown, but it is believed that it functions as a cellular adhesion molecule regulating synaptic plasticity and axonal growth. It also modulates signalling by catalytic TrkB, formation of heterodimers, and can also act as a negative modulator of BDNF signalling. It can inactivate BDNF released into the synapse and thus act as a reservoir for BDNF for later use (Lindsay et al., 1994). Binding of BDNF to its TrkB receptors activates several signalling pathways, such as the Ras/MAP kinase pathway, the PI kinase pathway, and the PLCγ pathway, by acting as a protein interaction site for these proteins.

In this study we found an association between the down-regulation of BDNF and the full-length TrkB receptors, such that the protein expression of both was down-regulated in the PFC of teenage suicide victims, but only the protein expression of TrkB receptors, and not of BDNF, was down-regulated in the hippocampus. This observation thus suggests decreased BDNF-mediated signalling in the teenage suicide brain.

Another intriguing finding was of a disassociation between the changes in the protein and in the mRNA expression of BDNF in the hippocampus, since we found a decrease in the mRNA but not in the protein expression of BDNF in the hippocampus of teenage suicide victims. Such an unpredictable relationship between protein and mRNA expression has been observed before: BDNF mRNA and protein expression levels have been shown to be regulated independently following seizure activity in the hippocampus (Wetmore et al., 1994) and during the development of the visual system in children (Johnsson et al., 1997), as has been discussed by McAllister et al. (1999).

There are some reports suggesting independent regulation of BDNF and TrkB receptors, for example, Nibuya et al. (1995) found that, although immobilisation stress decreased the expression of BDNF mRNA, it caused up-regulation of catalytic TrkB mRNA. Schaaf et al. (1997) found that administration of corticosterone decreased BDNF mRNA expression in the rat hippocampus but increased TrkB mRNA expression at low doses of corticosterone yet had no effect at higher doses. Although it is hard to speculate why BDNF and TrkB are co-regulated in the cortex but not the hippocampus, it is probable that TrkB receptor down-regulation is regulated by low BDNF in the cortex but may be independently regulated in the hippocampus.

As discussed earlier, a major risk factor for suicidal behaviour is the presence of depression or other mental disorders. Since abnormalities of BDNF and TrkB have been suggested in MDD and BP illness, we examined for a difference in BDNF and TrkB levels between the various diagnostic groups of our subjects, such as MDD, conduct or adjustment disorders, and no mental illness. However, we found no significant differences. These results may thus suggest that the observed changes in BDNF and TrkB in teenage suicide victims may be independent of diagnosis. The characteristics, risk factors, or the pathophysiology of teenage suicide and depression may be subtly different from those of adult suicide, as has been discussed by several investigators (Apter et al., 1995; Brent et al., 1993). One of the major differences between adolescent and adult depression is that adolescent depression is non-responsive to classical antidepressants but responds to treatment with SSRIs (Kaufman et al., 2001). In this study of BDNF and TrkB in teenage suicide, we have also found similarities, as well as subtle differences, between teenage and adult suicide.

We previously reported a study of BDNF and TrkB in the post-mortem brain from adult suicide victims (Dwivedi et al., 2003b). Whereas our findings of BDNF and TrkB in the PFC of teenage suicide victims are similar to those we reported in adults, decreases
in both mRNA and protein levels of BDNF were observed in the hippocampus of adult suicide victims, but a decrease in BDNF mRNA, and not in BDNF protein, was observed only in teenage suicide victims. Whether this subtle difference represents differences in some characteristics between teenage and adult suicide victims is not clear.

Teenage suicide brain vs. adult suicide brain

The suggestion that the neurobiological abnormalities associated with teenage suicide may be different than those of adult suicide is based on the following observations. (1) The risk factors and the characteristics of teenage suicide may be different in some respects from those of adult suicide. For example, teenage suicide is driven more by impulsive aggressive behaviour and stress. Therefore, conduct and adjustment disorders may be one of the major risk factors for teenage suicide as opposed to the risk factors for adult suicide (Apter et al., 1995; Brent et al., 1993). (2) Depression or mood disorders are major risk factors for both teenage and adult suicide, and although there are similarities in the clinical picture and the longitudinal course of mood disorders in children, adolescents, and adults (Kovacs, 1996), there are subtle differences in the neurobiological correlates and the treatment response of depressed patients in these different age cohorts (Kaufman et al., 2001).

Because of the above reasons, we have studied the neurobiology in general and signal transduction systems in particular in the post-mortem brains of both teenage as well as adult suicide victims and have found interesting differences. For example, we found that the protein kinase A (PKA) activity was decreased in the PFC of both teenage and adult suicide victims, and in the hippocampus of the adult but not teenage suicide victims (Dwivedi et al., 2004; Pandey et al., 2005). We also found different protein and mRNA expression of PKA subunits (e.g. PKA-RIα and PKA-RIβ were decreased in the PFC of teenage suicide victims, whereas RIIβ and Cβ were significantly decreased in the PFC of adult suicide victims compared with normal control subjects). Similarly we found a decrease in the cyclic AMP response element (CRE)-DNA binding activity, as well as in the protein and mRNA expression of the cyclic AMP response element binding protein (CREB) in the PFC but not in the hippocampus of teenage suicide victims (Pandey et al., 2007), whereas these were significantly decreased in both the PFC and hippocampus of adult suicide victims (Dwivedi et al., 2003a). In contrast to the current findings, a decrease in BDNF and TrkB was found in both the PFC and hippocampus of adult suicide victims (Dwivedi et al., 2003b). In summary, therefore, it appears that abnormalities of these signalling molecules may occur both in the PFC and hippocampus of adult suicide victims, whereas the abnormalities may in general be restricted to the PFC of teenage suicide victims. Moreover, the abnormalities of either their isozymes or subunits may be different between the two groups. There are no other neurobiological studies of the post-mortem brain from teenage suicide victims reported in the literature. Whether these differences are related to the subtle differences in the risk factors or the characteristics of teenage vs. adult suicide victims is not clear at this time, but further research may clarify the differences between these two groups.

In summary, the present study indicates a dysregulation of both BDNF and TrkB protein and mRNA expression in the PFC of teenage suicide victims. This dysregulation appears to be related to suicide and not to the presence of a mental disorder, and thus may also be related to acute or chronic stress, a risk factor for suicidal behaviour. The consequences of these abnormalities are not clear, but the abnormality in BDNF/TrkB signalling may eventually lead to structural abnormalities in the brains of these patients due to its effects on apoptotic pathways. These BDNF/TrkB abnormalities thus may also represent a vulnerability to suicide and/or mental disorders.

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Statement of Interest

None.
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