Serum levels of BDNF are associated with craving in opiate-dependent patients

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Original Paper

Serum levels of BDNF are associated with craving in opiate-dependent patients

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Abstract

Preclinical study results suggest that brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) are involved in the modulation of addictive behaviour. We investigated alterations in serum levels of BDNF and GDNF in opiate-dependent patients (28 males) who received diacetylmorphine treatment within a structured opiate maintenance programme. BDNF ($T = 2.735$, $p = 0.009$) serum levels were significantly increased in the opiate-dependent patients as compared with healthy controls (21 males), whereas GDNF serum levels ($T = 1.425$, $p = 0.162$) did not differ significantly from GDNF serum levels of the healthy controls. BDNF serum levels were significantly associated with craving for heroin (measured by the Heroin Craving Questionnaire ($r = 0.420$, $p = 0.029$) and by the General Craving Scale ($r = 0.457$, $p = 0.016$), whereas GDNF serum levels were not associated with psychometric dimensions of heroin craving. In conclusion, our results show a positive association between BDNF serum levels and opiate craving in opiate-dependent patients.

Keywords

BDNF, craving, GDNF, neurotrophic growth factors, opiate dependence, opiate maintenance

Introduction

The activation of mesolimbic and mesocortical dopamine neurons is thought to represent a key mechanism underlying the addictive properties of drugs of abuse (Spanagel and Weiss, 1999). In this context, opiates are presumed to reduce GABAergic inhibitory effects on accumbal dopaminergic cells in the ventral tegmental area (VTA) by activation of μ-opioid receptors in GABAergic neurons (Johnson and North, 1992).

The brain-derived neurotrophic factor (BDNF) and the glial cell line-derived neurotrophic factor (GDNF) are neurotrophic neuropeptides that are well known to stimulate neuronal growth and differentiation and facilitate survival in developing neurons (Ghitza et al., 2010). Both neuropeptides have been reported to ensure trophic support to adult dopaminergic neurons and to be involved in the regulation of midbrain dopamine release (Akaneya et al., 1995; Ducray et al., 2006). Moreover, BDNF and GDNF have been linked to learning and memory formation. Both neuropeptides have been reported to be involved in long-term potentiation of synaptic strength, a mechanism that is thought to underlie both natural adaption mechanisms (Klein et al., 2010; Pedersen et al., 2010) and the development of addictive behaviour (Lu et al., 2004). In particular, BDNF and GDNF have been reported to potentiate cocaine seeking in cocaine-withdrawn rats following a period of cocaine self-administration (Lu et al., 2009) by reinforcing drug-related neuroadaptions within the VTA. Moreover, an increase of BDNF mRNA expression within the nucleus accumbens was linked to cocaine self-administration and relapse (Graham et al., 2007).

In rats, BDNF mRNA expression was reported to increase within the locus coeruleus after precipitated morphine withdrawal (Numan et al., 1998). Moreover, the single-nucleotide Val/Val polymorphism of the *BDNF* gene, which leads to enhanced intracellular trafficking and release of BDNF compared to the Met/Met polymorphism, was reported to be

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predominant in patients suffering from heroin dependence (Cheng et al., 2005).

As shown in preclinical studies, GDNF is secreted within the striatum and retrogradely transported to the dopaminergic neurons within the mesencephalon (Tomac et al., 1995). This retrograde GDNF signalling is thought to represent a negative feedback mechanism that explains the negative association between the central GDNF expression and addictive behaviour, as observed in a number of preclinical studies (Niwa et al., 2007). For example, chronic exposure to morphine was reported to be associated with a significant decrease of phosphoRet, the proteinkinase that mediates GDNF signalling (Messer et al., 2000). Moreover, increased striatal dopamine levels have been reported in mice with endogenously reduced GDNF levels (Airavaara et al., 2004). Consistent with the negative association between GDNF and central dopamine release, reduced levels of GDNF in heterozygous GDNF (+/−) knockout mice were reported to accelerate the tolerance to morphine-induced increase of locomotor activity, to increase morphine-induced psychomotor sensitization and to slightly enhance accumbal dopamine output following a small dose of morphine (Airavaara et al., 2007).

Moreover, supporting the putative role of GDNF in synaptic plasticity and learning processes, decreased GDNF expression in heterozygous GDNF (+/−) knockout mice was associated with a shorter duration of morphine-induced place preference (Airavaara et al., 2004).

Clinical evidence investigating a putative association between BDNF and GDNF serum levels and the symptomatology of substance dependence is rather scarce. Regarding opiate dependence, Angelucci et al. (2007) reported on decreased BDNF serum levels in opiate dependent patients.

Based on these study results, it was our goal to investigate (1) alterations in the BDNF and GDNF serum levels in opiate-dependent patients and (2) a putative association between the BDNF and GDNF serum levels and craving for opiates.

Materials and methods

We investigated 27 opiate-dependent male patients (mean age: 41.15 years, SD 6.50 years, mean duration of opiate dependence: 21.48 years, SD 6.25 years, mean dose of diacetylmorphine (DAM) injected: 328.15 mg/day, SD 121.72 mg/day, body mass index (BMI): mean 25.83, SD 4.60) recruited from the Heroin Prescription Center of the Psychiatric Hospital of the University of Basel (Table 1). All patients fulfilled diagnostic criteria of opiate dependence according to ICD-10 (International Classification of Diseases, 10th revision) and DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) and had participated in a DAM maintenance programme for at least two months before participating in the study. All patients received individual doses of injectable DAM (administered intravenously or intramuscularly) twice a day. Additionally, 8 patients received oral methadone (mean: 25 mg, SD 14.39 mg) and 7 patients oral DAM treatment (mean: 300 mg, SD 173.21 mg). Patients suffering from axis-one diagnoses other than opiate dependence and substance abuse were excluded from the study, as were patients showing positive breath alcohol concentrations. The study adhered to the Declaration of Helsinki and was approved by the Ethics Committee of the University of Basel. All patients gave written informed consent.

The BDNF and GDNF serum levels were investigated directly before and 45 minutes after regular injection of DAM in the morning (time frame of last injection 16 hours) and in the afternoon (7 hours after the morning injection). The BDNF and GDNF serum levels obtained in the opiate-dependent patients were compared to the BDNF and GDNF serum levels of healthy controls (21 healthy male controls, mean age: 37.67 years, SD 16.56 years, mean BMI: 24.09, SD 3.82). Controls were screened for alcohol abuse using the alcohol use disorder identification test/alcohol consumption questions (AUDIT-C) (Saunders et al., 1993) as well as for axis-one diagnoses using a structured interview. A score below 4 points was required for subjects to be included in the control group. Controls were negative for axis-one diagnoses according to ICD-10 or DSM-IV. Controls and patients investigated were of Caucasian race.

Craving for heroin was measured psychometrically using the Heroin Craving Questionnaire (HCQ) (Tiffany et al., 2000). The HCQ measures craving for heroin in terms of Desire, Intention to Use, Anticipation of Positive Outcome, Relief and Lack of Control.

Craving for heroin, methadone, alcohol and cocaine was additionally measured using a visual analogue scale of 100 mm length, on which the participants rated their actual intensity of opiate craving from 0 = no craving to 100 = very strong craving (General Craving Scale (GCS)).

The BDNF and GDNF plasma levels were assessed using the DuoSet enzyme-linked immunosorbent assay (ELISA) Development System (DY248, DY212 E, R&D Systems, Wiesbaden-Nordenstadt, Germany). All the assays were performed according to the manufacturer's directions. The lower

Table 1. Demographic data of heroin-addicted patients and healthy controls

	Heroin-addicted patients ($n = 27$)	Healthy controls ($n = 21$)
Mean age	41.15 years (SD 6.50 years)	37.67 years (SD 16.58 years)
Mean body mass index	25.83 (SD 4.60)	24.09 (SD 3.82)
Range of age	28–54 years	18–68 years
Age of first use	19.67 years (SD 5.36 years)	NA
Duration of drug use	21.48 years (SD 6.25 years)	NA
Average daily dose of diacetylmorphine	328.15 mg (SD 121.72 mg)	NA

NA, not applicable.

thresholds of determination were 21 pg/mL (BDNF) and 26 pg/mL (GDNF). The intra-assay and interassay coefficients of variation were 5.0% and 8.7% (BDNF) and 7.0% and 10.3% (GDNF), respectively.

Statistical analyses

BDNF serum levels were normally distributed according to the Kolmogorov–Smirnov test. GDNF serum levels were ln-transformed to reach normal distribution. Correlations between the BDNF and the ln-transformed GDNF serum levels and the psychometric dimensions of heroin craving were calculated using Pearson's correlation coefficient. Differences between the BDNF and the ln-transformed GDNF serum levels of the opiate-dependent patients and the healthy control group were calculated using the *t*-test for independent samples. Acute alterations of the BDNF and GDNF serum levels before and after the injection of DAM were assessed using the *t*-test for dependent samples. The data was analyzed using PASW Statistics 18.0 and Graph Pad Prism™ 5.0 (Graph Pad Software Inc., San Diego, CA).

Results

The BDNF and GDNF serum levels were not associated with the duration of opiate dependence, age, or dose of DAM injected (data not shown).

We found significantly increased BDNF serum levels in the opiate-dependent patients as compared with the healthy controls before DAM injection in the morning ($T = 2.735$, $p = 0.009$) and in the afternoon ($T = 2.893$, $p = 0.006$, see Figure 1 for details). The GDNF serum levels were not significantly changed compared to the serum levels of the healthy controls, either before DAM injection in the morning ($T = 1.425$, $p = 0.162$) or in the afternoon ($T = 1.851$, $p = 0.105$).

Comparison of the BDNF and GDNF serum levels before and after injection of the regular dose of DAM showed no significant differences in the morning (BDNF: $T = 0.273$, $p = 0.787$, GDNF: $T = 0.587$, $p = 0.563$) or in the afternoon (BDNF: $T = 1.877$, $p = 0.073$, GDNF: $T = 0.906$, $p = 0.374$).

The BDNF serum levels measured before the injection of the morning dose of DAM were significantly associated with the total score of the HCQ (mean: 238.80 points, SD 35.29, $r = 0.420$, $p = 0.029$), the subscale of the HCQ measuring desire for heroin (mean: 29.29 points, SD 8.51, $r = 0.412$, $p = 0.033$), and the score obtained on the GCS measuring heroin craving (mean: 71.30, SD 22.00, $r = 0.457$, $p = 0.016$).

The GDNF serum levels were not significantly associated with the psychometric dimensions of heroin craving (data not shown).

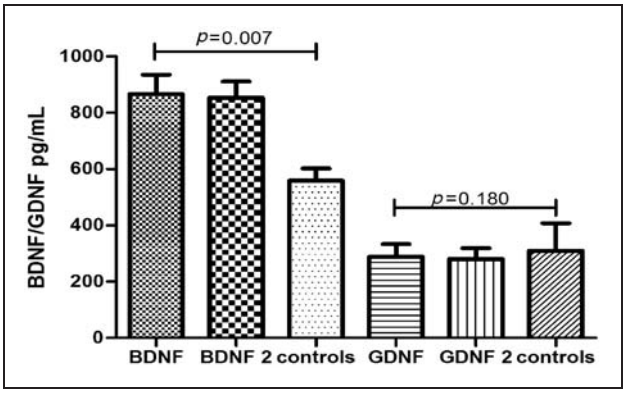

Figure 1. Means and SEMs of brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) serum levels before (BDNF, GDNF) and after (BDNF 2, GDNF 2) the scheduled injection of diacetylmorphine in opiate-dependent patients compared to healthy controls.

Discussion

In this pilot study, we found increased serum levels of BDNF in the opiate-dependent patients during opiate maintenance, whereas the GDNF serum levels were not significantly changed as compared to healthy controls.

Moreover, we found a significant association between the increased BDNF serum levels before the regular morning injection of DAM and craving for heroin. The GDNF serum levels were not associated with psychometric dimensions of heroin craving.

Our results regarding BDNF are consistent with observations obtained in preclinical studies suggesting a vice-versa modulation of the BDNF expression and the dopaminergic neurotransmission. For example, in rats the BDNF mRNA expression was found to be significantly increased following acute treatment with cocaine (Filip et al., 2006). Additionally, results obtained in behavioural studies suggested that BDNF increases a drug's rewarding effect by increasing the activity of nigrostriatal circuits and dopamine release (Altar et al., 1992). With respect to these results the increase of the BDNF serum levels in the opiate dependent patients observed in this study may be explained by opiate consumption.

There is broad evidence suggesting that GDNF is a negative modulator of drug intake. In particular, various studies report both increased self-administration and increased conditioned-place preference following cocaine, methamphetamine, alcohol and morphine intake in animal paradigms in which the GDNF function was decreased (for review, see Ghitza et al., 2010). In preclinical studies, chronic exposure to cocaine and morphine is consistently reported to cause a significant decrease in central GDNF signalling (Messer et al., 2000). Contrary to these preclinical study results, we found no significant difference between the GDNF serum levels of the healthy controls and opiate-dependent patients.

To this day, regarding GDNF, a putative association between peripheral blood levels and central GDNF expression has not been proved by preclinical data. Rather, recent studies have shown directly opposed GDNF levels in the cerebrospinal fluid and the serum of patients suffering from Alzheimer's disease (Straten et al., 2009), indicating a possible mismatch between the central and the peripheral GDNF levels.

However, regarding BDNF, there is preclinical evidence which suggests that the peripheral blood levels of BDNF

are correlated with central BDNF expression (Karege et al., 2002; Klein et al., 2010). Moreover, this association seems to be consistent throughout various species (Klein et al., 2010), which supports the possible role of BDNF serum levels as a biomarker for substance dependence.

In this way, the results obtained in this pilot study may point towards a potential usefulness of BDNF serum levels in the diagnosis and treatment of opiate dependence. Though, there are various factors that may limit the meaning and the interpretability of BDNF serum levels: study results show that depressive mood, antidepressant treatment, psychostimulants, nicotine consumption and gonadal hormones affect the peripheral blood levels of BDNF (Bus et al., 2011; Heberlein et al., 2010).

The impact of third-class factors like these may also explain the diverging results reported by Angelucci et al. (2007), who found decreased serum levels of the neurotrophic neuropeptides nerve growth factor (NGF) and BDNF compared with healthy controls in opiate-dependent patients. In this way, the associations observed in this pilot study may be corroborated by the low number of patients investigated as well as by third-class factors that have influence on the serum levels of neurotrophic neuropeptides.

In summary, our results show increased BDNF serum levels in the opiate-dependent patients that are associated with craving for heroin, although the underlying neurobiological mechanisms are not well understood. Further studies investigating BDNF serum levels in a greater number of opiate dependent patients are necessary to evaluate the potential role of BDNF as a biomarker of opiate craving and opiate intake as suggested by the preliminary results obtained in this pilot study. Follow-up studies may also determine a possible interaction between the BDNF serum levels and the BDNF levels in the cerebrospinal fluid and may thereby bolster our understanding of the peripheral function of neurotrophic neuropeptides and their potential usefulness as additional biomarkers in the diagnosis and treatment of substance dependence.

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