Decreased cerebrospinal fluid secretogranin II concentrations in severe forms of bipolar disorder

Joel Jakobsson, PhD; Mats Stridsberg, MD, PhD; Henrik Zetterberg, MD, PhD;
Kaj Blennow, MD, PhD; Carl-Johan Ekman, MD, PhD (candidate);
Anette G.M. Johansson, MD, PhD; Carl Sellgren, MD, PhD (candidate);
Mikael Landén, MD, PhD

Jakobsson, Zetterberg, Blennow, Landén — Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy, University of Gothenburg, Gothenburg and Mölndal, Sweden; Stridsberg — Department of Medical Sciences, Uppsala University, Uppsala, Sweden; Zetterberg — UCL Institute of Neurology, Queen Square, London, England; Ekman, Johansson, Landén — Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden; Sellgren, Landén — Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

Background: Bipolar disorder is a common psychiatric mood disorder that is defined by recurrent episodes of abnormally elevated mood and depression. Progressive structural brain changes in individuals with bipolar disorder have been suggested to be associated with defects in the secretion of neurotrophic factors. We sought to assess how the regulated secretory pathway in the brain is affected in patients with bipolar disorder by measuring chromogranin B and secretogranin II, which are 2 cerebrospinal fluid (CSF) biological markers for this process. **Methods:** We measured the concentrations of chromogranin B (peptide 439–451) and secretogranin II (peptide 154–165) in the CSF of patients with well-defined bipolar disorder and healthy controls. The lifetime severity of bipolar disorder was rated using the Clinical Global Impression (CGI) scale. **Results:** We included 126 patients with bipolar disorder and 71 healthy controls in our analysis. Concentrations of secretogranin II were significantly lower in patients with bipolar disorder type I than in healthy controls. The reduction was most pronounced in patients with high CGI scores (i.e., severe disease). **Limitations:** The cross-sectional design of the current study limits the ability to pinpoint the causalities behind the observed associations. **Conclusion:** This study shows that the CSF marker secretogranin II has the potential to act as a biological marker for severe forms of bipolar disorder. Our findings indicate that patients with bipolar disorder possess defects in the regulatory secretory pathway, which may be of relevance to the progressive structural brain changes seen in those with severe forms of the disease.

Introduction

Bipolar disorder is a common psychiatric mood disorder that is defined by recurrent episodes of abnormally elevated mood and depression.[1,2] Although the neurobiology of bipolar disorder is mainly unknown, there is growing evidence of progressive structural brain changes in affected individuals that might be related to the number of manic episodes.[3,4] The origins of these processes are unknown but might involve inflammation, changes in neurotrophins, mitochondrial dysfunction and oxidative stress.[3] The role of neurotrophins in disease progression and mood state in individuals with bipolar disorder has mainly been studied by analyzing peripheral (i.e., serum) concentrations of brain-derived neurotrophic factor (BDNF). Although there are discrepancies among studies, it appears that the concentration of peripheral BDNF is normal during euthymia and decreased during mood states (depression or mania; for a review see Fernandes and colleagues[5]). Altered concentrations of 2 other neurotrophins, neurotrophin-3 and neurotrophin 4/5, have also been reported in studies of patients with bipolar disorder.[6,7]

Neurotrophins, neuropeptides and hormones are in

neuroendocrine and endocrine cells stored in dense-core secretory granules (DCGs), and are synthesized and released in response to stimulation, thus resembling the regulated secretory pathway in the brain.[8] The major components of DCGs are proteins from a protein family called granins (e.g., chromogranin and secretogranin). Granin expression correlates with granule biogenesis, indicating that a primary role of granins is to sort and pack DCGs.[9–11] However, granins are also prohormones that can be intra- and extragranularly proteolytically processed into various bioactive peptides.[12] The derived granin fragments are detectable in cerebrospinal fluid (CSF), thus making them attractive as potential biological markers of the neuronal regulatory secretory pathway.[13] Altered (mainly reduced) CSF concentrations of different granins have previously been reported in studies of neurologic disorders like Alzheimer disease,[14] multiple sclerosis,[15] amyotrophic lateral sclerosis,[16] frontotemporal dementia[17] and schizophrenia.[18] A role of chromogranins in psychiatric disorders has been further suggested by genetic[19–22] and postmortem brain studies of patients with schizophrenia.[23,24] Interestingly, in this context, lithium — the treatment of choice to stabilize mood in patients with bipolar disorder — has been reported to enhance expression of granins and secretion of DCGs.[25–27]

To further our understanding of the role of the regulated secretory pathway in the pathophysiology of bipolar disorder, we set out to analyze patients with bipolar disorder and healthy controls for fragments of 2 granin proteins in CSF, namely chromogranin B (CgB, peptide 439–451) and secretogranin II (SgII, peptide 154–165).

Methods

Participants

We recruited patients from the St. Göran bipolar project, enrolling patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic, Stockholm, Sweden. The work-up and diagnostic assessments have been described in detail previously.[28] The key clinical assessment instrument used was the Affective Disorder Evaluation (ADE), developed for the Systematic Treatment Enhancement Program of Bipolar Disorder.[29] The general inclusion criteria were that patients had to be at least 18 years old and meet the DSM-IV-TR criteria for bipolar disorder type I and type II. The information collected included the number of depressive, manic and mixed episodes and the history of psychosis. The lifetime severity of bipolar disorder was rated using the Clinical Global Impression (CGI) rating scales.[30] This 7-point scale reflects the clinician's rate of the severity: 1 = normal or not at all ill, 2 = borderline mentally ill, 3 = mildly ill, 4 = moderately ill, 5 = markedly ill, 6 = severely ill and 7 = extremely ill. To determine euthymia, the Montgomery–Åsberg Depression Rating Scale (MADRS)[31] and the Young Mania Rating Scale (YMRS)[32] were used (i.e., MADRS < 14 and YMRS < 14). Information on somatic comorbidities, which are common among patients with bipolar disorder, was also collected.[33] For ethical reasons, patients continued to take their prescribed medications at the time of CSF sampling.

Age- and sex-matched healthy, population-based controls were randomly selected by Statistics Sweden and contacted by mail. The work-up and exclusion criteria for the controls are described in detail in Appendix 1, available at cma.ca /jpn. In brief, controls underwent a psychiatric interview conducted by experienced clinicians (C.-J.E. and A.G.M.J.) using the Mini International Neuropsychiatric Interview to exclude individuals with psychiatric disorders.[34] Psychiatric interviews involving the Alcohol Use Disorders Identification Test and Drug Use Disorders Identification Test, as well as assessment of the serum levels of carbohydrate-deficient transferrin, were used to exclude substance abusers.[35] The controls completed the same investigations the patients had undertaken, including self-rating scales, somatic tests, blood tests and lumbar puncture.

The study was approved by the Regional Ethics Committee in Stockholm and conducted in accordance with the latest Helsinki Protocol. All enrolled patients and controls consented verbally and in writing to participate in the study. Informed consent was obtained during a euthymic period (i.e., during a time period when patients did not meet criteria for a depressive or manic episode). All patients were capable of freely giving fully informed consent, as determined by the physicians who enrolled the patients.

CSF sampling

Cerebrospinal fluid sampling (by lumbar puncture) was performed when the participants were in a stable euthymic mood. Patients fasted overnight before the CSF collection at 9 am. The spinal needle was inserted into the L3/L4 or L4/L5 interspace, and a total volume of 12 mL of the CSF was collected, gently inverted to avoid gradient effects, and divided into 1.0–1.6 mL aliquots that were stored at –80°C pending analysis. An identical procedure was performed for the controls. All samples in this study were thawed and refrozen once before analysis. The granins are stable proteins and are usually not affected by a few freeze and thaw cycles.

Chromogranin B and secretogranin II analysis

We measured CSF concentrations of CgB- and SgII-derived peptides with radioimmunoassays, which have been previously described.[36,37] For CgB, antisera directed against CgB residues 439–451 were used. For SgII, secretoneurin antisera directed against SgII residues 154–165 were used. All standards and samples were assayed in duplicates, and the total assay variation was less than 10%.

Statistics

We used SPSS Statistics version 20 (IBM Corp.) for the statistical analysis. Analysis of covariance (ANCOVA) was used to analyze patient–control differences, using sex and age as covariates, and multiple linear regression analysis ("enter" method) was used to identify biomarker concentration predictors within the bipolar patient group. All p values are presented as 2-tailed, and we considered results to be significant at $p < 0.05$.

Results

In this study, we included 126 patients with bipolar disorder and 71 healthy controls. Demographics, clinical characteristics and ongoing medications for this study population are described in Table 1. In this sample, 43 patients had 1 or more somatic comorbidities: diseases of the skin and subcutaneous tissue ($n = 10$); neoplasms ($n = 1$); endocrine, nutritional and metabolic diseases ($n = 9$); diseases of the musculoskeletal system and connective tissue ($n = 7$); diseases of the nervous system ($n = 4$); diseases of the digestive system ($n = 8$); diseases of the circulatory system ($n = 1$); diseases of blood and blood-forming organs ($n = 2$); diseases of the eye and adnexa ($n = 2$); diseases of the respiratory system ($n = 9$); diseases of the genitourinary system ($n = 1$); injury, poisoning and certain other consequences of external causes ($n = 1$); and "others" ($n = 6$).

We analyzed the CSF concentrations of 2 granin fragments, SgII (peptide 154–165) and CgB (peptide 439–451). There were no significant differences in CgB and SgII among patients with bipolar disorder (both type I and II) and controls (SgII: $F_1 = 2.982$, $p = 0.09$; CgB: $F_1 = 1.402$, $p = 0.24$). Next, we analyzed the granin concentrations between patients with bipolar disorder type I or type II and controls (Fig. 1). We found that the CSF concentrations of SgII were significantly lower in patients with bipolar disorder type I than in healthy controls (SgII: $F_1 = 4.719$, $p = 0.031$), whereas no significant differences were found in the CgB concentrations (CgB: $F_1 = 3.436$, $p = 0.07$). No differences in SgII or CgB were found between patients with bipolar disorder type II and controls (SgII: $F_1 = 0.331$, $p = 0.57$; CgB: $F_1 = 0.004$, $p = 0.95$, respectively).

We conducted a multiple linear regression analysis of the CgB and SgII concentrations in relation to medication groups and clinical characteristics within the patient group (7 patients were excluded owing to missing data). The significant predictors for SgII were CGI score ($\beta = -0.33$, $t = -3.21$, $p = 0.002$), lamotrigine ($\beta = 0.24$, $t = 2.51$, $p = 0.014$) and prior episode of psychosis ($\beta = 0.26$, $t = 2.09$, $p = 0.039$; regression

Table 1: Demographics and clinical characteristics of the study population

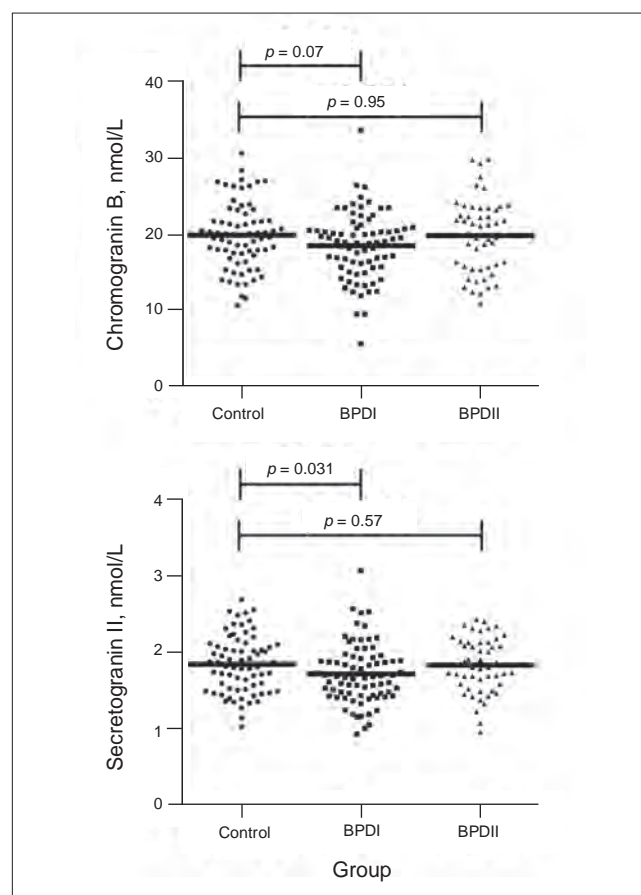| Characteristic	Control	Bipolar disorder
	Group; no (%)*	
Sex, male:female, no.	26:45 (37:63)	49:77 (39:61)
Education		
Not completed compulsory school	0 (0)	3 (2.2)
Completed compulsory school	0 (0)	12 (9.5)
Completed upper secondary school	25 (35.2)	33 (26.2)
Completed higher education < 3 yr	15 (21.1)	16 (12.7)
Completed higher education > 3 yr	31 (43.7)	62 (49.2)
Living condition†		
Alone	24 (33.8)	64 (50.8)
With partner	44 (62.0)	48 (38.1)
With parent/parents	2 (2.8)	8 (6.3)
With other adults	1 (1.4)	6 (4.8)
Primary source of income†		
Employment/education	69 (97.2)	67 (57.3)
Sickness benefit	1 (1.4)	43 (36.8)
Supplementary benefit	0 (0.0)	3 (2.6)
Relative's or own capital	1 (1.4)	4 (3.4)
Diagnosis		
Bipolar disorder type I		76 (60.3)
Bipolar disorder type II		50 (39.7)
Prior psychosis		65 (51.6)
Medications		
Lithium		75 (59.5)
Lamotrigine		27 (21.4)
Valproate		15 (11.9)
Antidepressants		58 (46.0)
Benzodiazepines		28 (22.2)
Atypical antipsychotics		29 (23.0)
Age, median (IQR) yr	32 (27–43)	34 (28–50)
Total no. episodes, median (IQR)		10 (6–22)
Clinical Global Impression,[30] median (IQR) score		4 (4–5)
YMRS, median (IQR) score		0 (0–1)
MADRS, median (IQR) score		1 (0–4)

IQR = interquartile range; MADRS = Montgomery–Åsberg Depression Rating Scale;[31]
YMRS = Young Mania Rating Scale.[32]
*Unless otherwise indicated.
†Data from 117 patients.

Fig. 1: Cerebrospinal fluid concentrations of chromogranin B (CgB) and secretogranin II (SgII) in healthy controls ($n = 71$), patients with bipolar disorder type I (BPDI, $n = 76$) and bipolar disorder type II (BPDII, $n = 50$). The mean is shown as a straight line. Analysis of covariance was used for comparisons between patients and controls (using age and sex as covariates).

model $R^2 = 0.203$). Lamotrigine was also significantly associated with CgB concentrations ($\beta = 0.22$, $t = 2.22$, $p = 0.028$; regression model $R^2 = 0.143$). Multiple linear regression statistics for all included predictor variables are provided in Appendix 1, Table S1 (SgII) and Table S2 (CgB).

Discussion

In this study, we assessed 2 biological markers of the regulatory secretory pathway in the brain, CgB and SgII, in CSF from patients with bipolar disorder and from sex- and age-matched healthy controls. We found significantly lower SgII concentrations in the CSF of patients with bipolar disorder type I than in controls. Moreover, post hoc analyses revealed that low CSF concentrations of SgII were associated with more severe illness (as defined by CGI score). Although only patients with bipolar disorder type I differed from controls, CGI score emerged as a good predictor for SgII independent of bipolar disorder diagnosis. Thus, SgII is a possible biological marker for disease severity in patients with bipolar disorder. We found that CgB did not differ significantly between patients and controls.

Low SgII concentrations in CSF may reflect generally reduced activity in the regulated secretory pathway. This might in turn reflect a reduced capacity in secretion of neuropeptides, neurotrophins and hormones specific to SgII-positive DCGs.[38] The 154–165 peptide of SgII is, however, part of the 33 amino acid peptide secretoneurin that has been reported to have multiple effects on its own (e.g., stimulating release of dopamine from negrostriatal neurons,[39,40] having angiogenic effects,[41] promoting neuroprotection and neuronal plasticity[42]). Thus, low SgII concentrations in patients with bipolar disorder might reflect decreased neuronal/synaptic plasticity and survival, which may in turn have impact on progressive brain changes and disease severity. Speculatively, defects in neurotrophin signalling might underlie progressive structural brain changes in patients with bipolar disorder.

Genetic and epidemiological studies suggest that bipolar disorder and schizophrenia share genetic vulnerability traits.[43–45] The diseases also share deficits in cognitive functions,[46,47] alterations in brain morphology[48,49] and response to antipsychotic medications,[50] suggesting that these disorders share many pathophysiological aspects. The diagnosis of bipolar disorder is mainly based on clinical assessments, and there is a lack of biological markers that, in addition to supplementing the diagnostic assessment, also could predict treatment response, side effects and/or disease progression.[51] It is interesting to note that our finding of decreased CSF levels of SgII in bipolar disorder contrasts with that from a previous study of patients with schizophrenia (unaltered concentrations of SgII).[18] Hypothetically, these contrasting findings might reflect a functional difference in the underlying pathogenesis of schizophrenia and bipolar disorder. However, psychosis was positively associated with SgII in the present study. Thus, it is possible that disease severity in patients with schizophrenia is associated with low concentrations of SgII as well and that the concentrations are counteracted by episodes of psychosis.

There was a positive association between lamotrigine and SgII (and CgB), indicating that lamotrigine might affect the regulatory secretion pathway. In line with this observation, previous studies have reported an upregulation of BDNF expression and secretion in response to lamotrigine treatment.[52,53] It should be noted, however, that in this cross-sectional case–control study, we were not able to rule out the possibility that higher CSF concentration of SgII is associated with a subset of patients with bipolar disorder treated with lamotrigine as opposed to other medication.[54]

Limitations

Case–control studies on CSF from patients with psychiatric disorders are generally limited by sample size, increasing the risk of type II errors. Identifying biological differences might yield important pathophysiological insights despite small effect sizes. Thus, a distinct strength in this study was the relatively large sample size, which was further reinforced by the accurate and comprehensive clinical evaluation of both patients and controls. No correction for multiple comparisons was performed in the post hoc multiple linear regression analyses. However, as we think that these findings give important insight into the pathophysiology of bipolar disorder and suggest directions for future studies, we chose to report original p values but stress that these findings should be interpreted cautiously. It is also important to note that the regression models only explained small portions of the variances. Another strength is that CSF, reflecting the brain chemistry more closely than serum, was available for patients and controls. However, the cross-sectional design of our study limits the ability to pinpoint whether a relatively low SgII concentration is a result of or basis of bipolar disorder severity. A longitudinal analysis of SgII together with CGI score assessments would adequately address this issue. Likewise, the effect of lamotrigine on the SgII concentrations should be tested longitudinally in a before-and-after treatment manner. Furthermore, it is important to verify the present findings in an independent sample population to rule out false-positive associations.

Conclusion

By measuring the CgB and SgII concentrations in CSF from patients with bipolar disorder and healthy controls, we found a lower concentration of SgII in patients, especially in those with severe disorder. As SgII may act as a neuroprotective peptide and is coreleased with neurotrophins, the findings support the hypothesis that defects in neurotrophin signalling is involved in the pathophysiology of severe bipolar disorder.

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