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ORIGINAL PAPER

Reduced density of hypothalamic VGF-immunoreactive neurons in schizophrenia: a potential link to impaired growth factor signaling and energy homeostasis

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Abstract Protein expression of VGF (nonacronymic) is induced by nerve/brain-derived growth factor, neurotrophin 3, and insulin. VGF is synthesized by neurons in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus. After enzymatic processing, smaller VGF-derived peptides are secreted into the cerebrospinal fluid (CSF) or blood. These peptides play important roles by improving synaptic plasticity, neurogenesis, and energy homeostasis, which are impaired in schizophrenia. Based on previous observations of neuroendocrine and hypothalamic deficits in schizophrenia and to determine whether increased levels of the VGF fragment 23-62 in CSF, which have been described in a recent study, were related to changes in hypothalamic VGF expression, an immunohistochemical study was performed in 20 patients with schizophrenia and 19 matched control subjects. N- (D-20) and C-terminal (R-15) VGF antibodies yielded similar results and immunolabeled a vast majority of PVN and SON neurons. Additionally, D20-VGF immunohistochemistry revealed immunostained fibers in the pituitary stalk and neurohypophysis that ended at vessel walls, suggesting axonal transport and VGF secretion. The cell density of D20-VGF-immunoreactive neurons was reduced in the left PVN ($P = 0.002$) and SON ($P = 0.008$) of patients with schizophrenia. This study provides the first evidence for diminished hypothalamic VGF levels in schizophrenia, which might suggest increased protein secretion. Our finding was particularly significant in subjects without metabolic syndrome (patients with a body mass index $\leq$28.7 kg/m^2). In conclusion, apart from beneficial effects on synaptic plasticity and neurogenesis, VGF may be linked to schizophrenia-related alterations in energy homeostasis.

Keywords Schizophrenia · Hypothalamus · Postmortem · Histopathology · VGF · Granins

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Introduction

The VGF (nonacronymic) gene encodes a secreted protein that is synthesized widely by neurons in the brain, spinal cord, and peripheral nervous system [10]. In addition, VGF was found in the hypophysis, adrenal medulla, gastrointestinal, and pancreatic endocrine cells, suggesting important neuroendocrine functions [10]. The VGF protein is

subsequently processed by the neuroendocrine-specific prohormone convertases PC 1/3 and PC 2 into more than 10 different peptides [21]. After the identification of the VGF protein in 1985 [20], subsequent studies have observed that VGF expression is induced by nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 and marginally by epidermal/fibroblast growth factor and insulin [4]. Within the hippocampus, VGF mRNA is expressed in CA1, CA2, and CA3, the hilus of the dentate gyrus and the subicular complex as well as layer II of the entorhinal cortex [28]. Recent studies suggest a role of VGF in animal models of depression [32]. According to these studies, particularly C-terminal VGF peptides are enhancing hippocampal synaptic plasticity as well as neurogenesis in the dentate gyrus in a dose-dependent manner [1, 32]. VGF may be likewise involved in the pathophysiology of schizophrenia, which is also associated with impaired synaptic plasticity [9] and neurogenesis [19, 24].

As mentioned above, VGF is synthesized widely by several neurons in the brain, but its expression is particularly abundant in the paraventricular (PVN) and supraoptic (SON) nuclei of the hypothalamus [36]. After enzymatic processing, smaller VGF-derived peptides are secreted into body fluids such as the cerebrospinal fluid (CSF) or blood [18]. These VGF peptides play an important role in energy homeostasis, synaptic function, neurogenesis, pain modulation and sexual behavior [for a recent review, see [10]]. Regarding energy homeostasis, previous studies have shown that VGF knockout mice are thin, small, hypermetabolic, hyperactive, and show reduced fertility with markedly reduced leptin levels and fat stores [38]. Moreover, in response to fasting, VGF mRNA levels are induced in the normal mouse hypothalamic arcuate nuclei [15].

A recent publication has suggested that VGF peptides may be useful biomarkers for neurological and psychiatric disorders, such as Alzheimer's diseases, frontotemporal dementia, and schizophrenia [3]. For example, the VGF fragment 23-62 (amino acids 23-62 of the native VGF protein) levels are increased in the CSF of two independent groups of patients suffering from first-onset, drug-naïve schizophrenia [17]. These CSF findings, the involvement of VGF in the regulation of energy metabolism and its predominant hypothalamic expression suggest a particular importance of VGF in schizophrenia. Changes in energy metabolism, such as an increased prevalence of "metabolic syndrome", which is characterized by visceral obesity, type 2 diabetes, elevated lipid levels, hypertension, and decreased sensitivity to insulin, have been identified as risk factors for the increased mortality rate of patients with schizophrenia [33, 39]. Previously, these metabolic disturbances were almost exclusively attributed to side effects of atypical antipsychotic medication, such as clozapine and olanzapine [23]. However, impaired fasting glucose tolerance has been reported in approximately 30% of all drug-naïve schizophrenia cases and is disease-inherent [13].

Notably, the hypothalamus is strongly involved in the pathophysiology of schizophrenia [5]. Psychotic episodes are evoked by stress, which activates the hypothalamic–pituitary–adrenal (HPA) axis. This is in line with the stress-vulnerability concept of schizophrenia, which postulates that vulnerability may include genetic predisposition and birthing complications—while stressful life events and biological stressors may exacerbate the illness as environmental factors by triggering the occurrence of symptoms [41]. Indeed, elevated baseline levels of cortisol and adrenocorticotropic hormone (ACTH) have been measured in patients with schizophrenia along with an increased release of corticotropin-releasing hormone (CRH) from PVN neurons [5]. Due to a lack of appropriate human and animal experiments, it is currently unknown whether there is a direct link between stress axis activation and alterations of VGF expression in schizophrenia.

Using immunohistochemistry, we investigated whether increased levels of VGF in the CSF of patients with schizophrenia, which have been described in a previous study [17], may be related to changes in neuronal VGF expression in the hypothalamus. Because VGF potentially influences energy homeostasis, we evaluated the association between the body mass index (BMI) and hypothalamic VGF immunoreactivity in schizophrenia and control subjects. A qualitative evaluation of VGF in the pituitary stalk and posterior pituitary was performed to verify the axonal transport and secretion of VGF to the neurohypophyseal capillary bed. The anterior (adenohypophysis) pituitary was analyzed to evaluate alternative pituitary sources of VGF.

Materials and methods

Human hypothalami

Postmortem brains were obtained from the Magdeburg brain bank in accordance with the Declaration of Helsinki and the local institutional review board. Written consent was obtained from the next of kin. The donors were 20 patients with schizophrenia (mean age 52 years; 10 men, 10 women) and 19 neuropsychiatric healthy control subjects (mean age 51 years; 10 men, 9 women). These cases were matched with respect to age, gender, and autolysis time (Table 1). All patients had received typical antipsychotic medication in the last 90 days prior to death.

Information for clinical diagnoses was obtained by the careful review of clinical records and structured interviews

Table 1 Demographic and histological data of patients with schizophrenia ($n = 20$) and healthy control subjects ($n = 19$)

Diagnosis (DSM-IV)	Gender	Age (years)	Autolysis (h)	BMI (kg/m^2)	CPZ (mg)	Cause of death
SCZ, paranoid type	m	65	66	14.2	250	Heart failure
SCZ, paranoid type	m	46	48	30.4	275	Pulmonary embolism
SCZ, paranoid type	m	50	72	26.7	650	Heart failure
SCZ, paranoid type	m	34	5	19.0	0	Suicide by hanging
SCZ, paranoid type	f	60	48	19.0	400	Heart failure
SCZ, paranoid type	f	53	48	n.a.	240	Myocardial infarction
SCZ, paranoid type	f	63	72	n.a.	0	Suicide by hanging
SCZ, paranoid type	f	38	12	n.a.	n.a.	Acute respiratory failure
SCZ, paranoid type	f	65	72	n.a.	n.a.	Heart failure
SCZ, paranoid type	f	55	48	n.a.	0	Suicide by intoxication
SCZ, paranoid type	f	59	48	35.9	750	Heart failure
SCZ, paranoid type	f	62	48	36.6	n.a.	Pulmonary embolism
SCZ, paranoid type	f	40	48	35.9	n.a.	Ileus
SCZ, residual type	m	39	12	24.4	n.a.	Heart failure
SCZ, residual type	m	51	48	20.5	1,200	Ileus
SCZ, residual type	m	50	48	24.4	475	Heart failure
SCZ, residual type	m	57	72	n.a.	400	Heart failure
SCZ, residual type	m	47	24	30.1	1,230	Heart failure
SCZ, residual type	m	48	48	29.7	1,000	Acute respiratory failure
SCZ, residual type	f	54	24	36.6	n.a.	Pulmonary embolism
SCZ cases (ratio/mean ± SD)	*10 m/10f*	*52 ± 9*	*45 ± 21*	*27.4 ± 7.4*	*490 ± 421*	
Control	m	56	48	21.6	0	Retroperitoneal hemorrhage
Control	m	50	72	n.a.	0	Myocardial infarction
Control	m	47	24	34.6	0	Myocardial infarction
Control	m	56	30	n.a.	0	Heart failure
Control	m	52	10	29.4	0	Heart failure
Control	m	40	72	n.a.	0	Myocardial infarction
Control	m	64	35	19.6	0	Ruptured aortic aneurysm
Control	m	61	24	26.5	0	Myocardial infarction
Control	m	54	24	n.a.	0	Pulmonary embolism
Control	m	46	24	21.6	0	Heart failure
Control	f	52	24	27.7	0	Heart failure
Control	f	48	48	17.3	0	Status asthmaticus
Control	f	64	24	14.3	0	Peritonitis
Control	f	33	72	20.7	0	Aortic embolism
Control	f	50	72	30.8	0	Ruptured aortic aneurysm
Control	f	30	48	n.a.	0	Pulmonary embolism
Control	f	64	26	n.a.	0	Myocardial infarction
Control	f	64	24	<18.5	0	Gastrointestinal hemorrhage
Control	f	38	24	29.4	0	Heart failure
Controls (ratio/mean ± SD)	*10 m/9f*	*51 ± 10*	*38 ± 20*	*24.0 ± 6.1*	*0 ± 0*	
Test	Chi-square	*t* Test	*t* Test	*t* Test		
Test value	$\chi^2 = 0.027$	$T = 0.235$	$T = 0.994$	$T = 0.800$		
P-value	0.869	0.801	0.327	0.434		

BMI body mass index, *CPZ* chlorpromazine equivalents of the mean daily dose of antipsychotic medication taken by the patients during the last 90 lifetime days, *f* female, *m* male, *n.a.* not available, *SD* standard deviation, *SCZ* schizophrenia

of physicians who were involved in the treatment or persons who lived with or had frequent contact with the subjects before death. The DSM-IV Axis I diagnosis was established in consensus meetings by two psychiatrists (HB and JS) using all available information from interviews and clinical records [2]. Brains with lifetime reports of substance abuse, dementia, neurological illness, severe trauma, or chronic terminal diseases that are known to affect the brain were excluded. Additionally, neuropathological changes due to neurodegenerative disorders, tumors, inflammatory, vascular, or traumatic processes were excluded following assessments by an experienced neuropathologist (CM). The determination of suicide was made by a forensic pathologist (TG) and was verified based on the individual records.

Tissue preparation was performed as previously described [7, 29, 30]. Briefly, the brains were fixed in 8% phosphate-buffered formaldehyde (pH 7.0) for 3 months. After separation of the brainstem and cerebellum, the hemispheres were divided by coronal cuts into three bi-hemispherical coronal blocks comprising the frontal lobe anterior to the genu of the corpus callosum ("anterior" block), the fronto-temporo-parietal lobe extending over the entire length of the corpus callosum ("middle" block) and the occipital lobe ("posterior" block). After embedding the brains in paraffin, serial coronal whole brain sections of 20 μm were cut and mounted. The actual thickness of the sections was determined by focusing the upper and lower surfaces of the section and subtracting the *z*-axis coordinate of the lower surface from that of the upper surface. The movements in the *z*-axis were measured using a microcator as an integral part of the Leica DM RB microscope Leica, Giessen, Germany). The section thickness after the histological procedures was 18.7 ± 1.1 μm (mean ± SD).

Human pituitary glands

Three hypophyses were obtained upon autopsy (kindly provided to the Magdeburg brain bank in accordance with the Declaration of Helsinki and the local institutional review board by Dr. K. Trübner, Department of Forensic Medicine, University of Essen, Germany). The samples came from two male subjects (aged 55 and 59 years, subjects died from generalized sepsis and suicide (hanging); postmortem intervals of 24 and 14 h, respectively) and one man (aged 33 years, subject was killed in a car accident; postmortem interval of 31 h) without psychiatric disorders. Pituitary glands were removed from the cranium, fixed in toto in 8% formalin, embedded in paraffin and cut into sections of 20 μm using a microtome. For morphological orientation, every eighth section was stained with Azan.

Western blot analysis

Frozen tissue samples from hypothalami were pulverized in liquid nitrogen and subsequently homogenized in lysis buffer (RIPA buffer, Sigma-Aldrich, Taufkirchen, Germany) containing a protease inhibitor cocktail (Merck, Darmstadt, Germany), 1 mM sodium orthovanadate and 0.1 M dithiotreitol (Sigma-Aldrich, Taufkirchen, Germany). Tissue homogenates were centrifuged (4°C, 14,000 rpm) for 10 min. The resulting supernatant was stored at −20°C until further use. Western blot analysis was done after blocking the polyvinylidene fluoride membrane (Whatman, Schleicher and Schuell, Dassel, Germany) with 5% low-fat milk in TBS + 0.1% Tween20 for 1 h. Aliquots representing 20 μg of protein were subjected to SDS–PAGE, transferred to a membrane and incubated with two different primary antibodies against VGF at dilutions of 1:500 for 24 h at 4°C: a polyclonal goat anti-VGF antibody against a peptide sequence near the N-terminus of VGF (sc-10381/D-20; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a polyclonal goat anti-VGF antibody against a peptide at the C-terminus of VGF (sc-10383/R-15; Santa Cruz Biotechnology, Santa Cruz, CA, USA). The membrane was washed four times in TBST buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, and 0.1% Tween 20). Secondary detection was performed using horseradish peroxidase-conjugated anti-goat immunoglobulin G (1:5,000; Santa Cruz). After four washings with TBST, horseradish peroxidase activity was visualized using the SuperSignal West Dura substrate (Pierce, Bonn, Germany) followed by exposure of the membrane to X-ray film. In addition, molecular mass protein markers (Biotinylated Protein ladder, Cell Signaling Technology, Danvers, USA) that were diluted to 1:5,000 in TBS were employed.

Immunohistochemistry

Formalin-fixed tissue sections were deparaffinized and antigen demasking was performed by boiling the sections for 4 min in 10 mM citrate buffer (pH 6.0). Preincubation with 1.5% H_2O_2 for 10 min to block endogenous peroxidase activity was followed by 10% normal goat serum for 60 min to block nonspecific binding sites and repeated washings with PBS. The two different polyclonal VGF antibodies mentioned above were employed at a dilution of 1:200 for 72 h at 4°C. The sections were incubated for 2 h at room temperature with a biotinylated swine anti-goat secondary antibody (Multi-link, E 0453; Dako, Glostrup, Denmark) for the application of the streptavidin–biotin technique. The chromogen 3,3′-diaminobenzidine (DAB) and ammonium nickel sulfate were used to visualize the reaction product [16]. The specificity of the

immunoreactions was controlled by the application of buffer instead of primary antiserum.

In pilot studies, we first had compared both the C-terminal and the N-terminal antibodies with regard to their immunohistochemical staining properties by alternately immunostaining consecutive sections through the human hypothalamus (from three control brains and one brain of an individual with schizophrenia). These sections were 20 μm thick. Immunostained magnocellular PVN and SON neurons having diameters larger than 20 μm were directly identifiable on two neighboring sections. Thus, we could see whether the two antibodies had stained identical neurons. However, parvocellular neurons (having diameters below 20 μm) appeared only on one of the two neighboring sections. Hence, we counted the number of all VGF-immunostained hypothalamic neurons on all sections and found (nearly) identical neuron numbers on neighboring sections, of which one was immunostained with the N-terminal antibody and the other with the C-terminal one.

From these results, we concluded that both antisera recognize identical hypothalamic cell populations. However, during these experiments, it also came out that the D20-antibody (directed against the N-terminus of VGF) generates a better signal-to noise ratio (which means, hypothalamic neurons were more strongly stained and the background staining was reduced). This was an argument to use the D20-antibody for the systematic study of 20 schizophrenia and 19 control subjects.

Morphometric analysis

The sections were selected at anatomical levels of the optic chiasm (Ox). The PVN and SON of the hypothalamus were delineated as illustrated in Fig. 2a [22, 27].

Cell counting of VGF immunoreactive neurons was performed in two coronal sections per brain at 100× magnification using the optical dissector method [6]. The left and right hemispheres were separately evaluated. Because the actual thickness of the sections was 18.7 ± 1.1 μm (mean ± SD), two well-defined optical planes within the section were used (distance of 16 μm between the upper and the lower guard zone). Immunostained cells that came into focus between the upper and lower optical plane were counted. After determining the number of cell profiles within this "counting box" (i.e., between planes of the dissector), the volume shrinkage factor of the brain and the area of the counting grid (1 mm × 1 mm = 1 mm^2), we calculated the number of VGF-immunoreactive cells within a given tissue volume (cell density). To establish interrater reliability between investigators, repeated measurements were performed by different investigators (SB and BJ). Intraclass correlation analysis yielded highly corresponding results ($r = 0.94$).

Statistical analysis

Statistical analyses were performed with the SPSS 15.0 program (Statistical Product and Service Solutions, Chicago, IL, USA). Given the normal distribution of the data, which was indicated by Kolmogorov–Smirnov tests, parametric tests such as *t* tests, analysis of variance (ANOVA), and the Pearson's correlation coefficient were employed. The demographic data were compared by Chi-square and *t* tests. A BMI >28.7 kg/m^2 has been previously described as a suitable predictor for the presence of metabolic syndrome in patients with schizophrenia [34]. Therefore, the diagnostic groups were split into two subcohorts (BMI >28.7 kg/m^2 versus BMI ≤28.7 kg/m^2), and the density of VGF-immunoreactive neurons was compared by ANOVA and post hoc *t* tests. The Pearson's correlation coefficients were calculated for the whole group as well as separately for the schizophrenia and control cohorts. Using these coefficients, we did not observe any influence of age, duration of disease, autolysis time, and medication dosage (chlorpromazine equivalents) on VGF-immunoreactive cell densities. Moreover, ANOVA revealed no influence of gender on the VGF results. Bonferroni correction was applied to control for type I errors.

Results

Western blot analysis

As illustrated in Fig. 1, the western blot analysis of VGF in human hypothalamic tissue showed three single bands with molecular weights of approximately 90, 60, and 34 kDa after the application of the N-terminal antibody (D-20) and three bands with molecular weights of 90, 60, and 45 kDa after the application of the C-terminal antibody (R-15). The tested antibodies detected prominent 90 and 60 kDa bands. The 90 kDa protein corresponds to intact VGF. All other bands are proteolytic cleavage products.

In addition to the abovementioned immunohistochemical pretests ("Immunohistochemistry"), these blotting results were a strong argument to use the N-terminal antibody (D-20) for the systematic immunohistochemical study ("Immunohistochemistry and morphometric analysis"), because more intact (and thus authentic) VGF is detected with this antibody compared with the C-terminal one. This is in full agreement with data of others, showing that the degradation of VGF starts from the C-terminus [10].

Immunohistochemistry and morphometric analysis

Typical staining results with the N-terminal antibody (D-20) in PVN and SON neurons are illustrated in Fig. 2b, c.

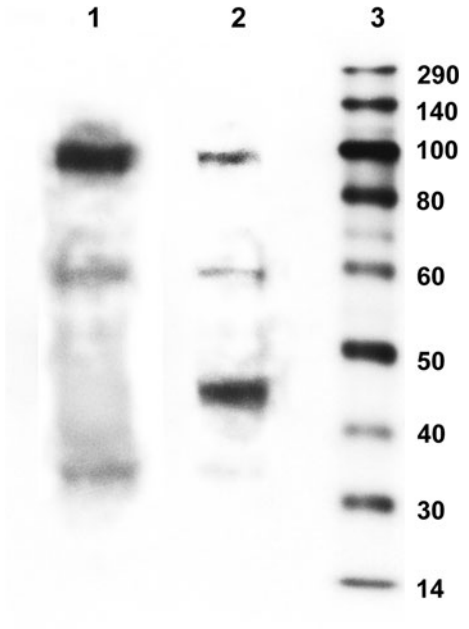

Fig. 1 Western blot analysis of VGF in human hypothalamic tissue. *Lane 1* shows the blot after application of the N-terminal antibody (D-20). VGF immunoreactivity was identified as three single bands with molecular weights of approximately 90, 60, and 34 kDa. *Lane 2* shows the blot after application of the C-terminal antibody (R-15). VGF immunoreactivity was identified as three bands with molecular weights of 90, 60, and 45 kDa. The bands in *lane 3* indicate the molecular mass markers. *Annotation:* The 90 kDa band corresponded to the intact molecule. All other bands were proteolytic cleavage products

We observed similar staining patterns with the C-terminal antibody (R-15; Fig. 2d). These data were confirmed using a comparative quantitative analysis of 6 different brains with D-20 and R-15 antibody staining, which yielded a correlation coefficient of 0.92.

The subsequent systematic analysis of schizophrenia and control subjects was performed using the D20-VGF-antibody. The analysis of VGF expression in the pituitary stalk and neurohypophysis (Fig. 2e) showed VGF-immunopositive fibers in these regions that ended at vessel walls of the posterior pituitary. However, as illustrated in Fig. 2f, VGF was detected in a subpopulation of epithelial cells in the adenohypophysis, indicating alternative sources of VGF.

A quantitative analysis of the D20-VGF immunostaining was performed in the PVN and SON. We observed that the cell density of VGF-immunoreactive neurons was reduced in the left PVN ($T(1, 30.7) = -3.361$, $P = 0.002$) and SON ($T(1, 30.8) = -2.859$, $P = 0.008$) of patients with schizophrenia compared with that in controls (Fig. 3). No significant diagnosis-related differences were observed in the right hemisphere (PVN: $T(1, 30.3) = -1.479$, $P = 0.149$; SON: $T(1, 30.4) = -0.996$, $P = 0.327$).

The reported effects were controlled for the potential confounding factors of age, gender, duration of disease, autolysis/fixation time, BMI, and medication dosage. Regarding the influence of BMI, the group difference between control and schizophrenia subjects with a BMI $\leq$28.7 kg/m^2 reached statistical significance (left hemisphere: $T(1, 6.34) = 2.937$, $P = 0.024$, right hemisphere: $T(1, 7.50) = 3.703$, $P = 0.007$). No significant differences were detected between control and schizophrenia subjects with a BMI >28.7 kg/m^2.

Discussion

VGF is a polypeptide precursor encoding different physiologically active neuropeptides and many VGF-derived fragments [10]. After comparative analyses of the R-15 and D-20 antibodies, we used the D-20 antibody that is raised against a peptide mapping near the N-terminus [35], to analyze the density of VGF-positive neurons in the hypothalamus of patients with schizophrenia and control subjects. Consistent with previous reports on the roles of the hypothalamus and the neuroendocrine system in schizophrenia [5, 13] and based on findings of a predominant hypothalamic expression of VGF [36], this study provides the first evidence for reduced neuronal VGF in the PVN and SON of patients with schizophrenia (Fig. 3). In accordance with a recent publication on VGF in the CSF of schizophrenia cases [17], our observation of decreased intracellular content of VGF may reflect increased VGF protein secretion from hypothalamic neurons. Indeed, we detected VGF-immunopositive fibers in the pituitary stalk and neurohypophysis. These fibers ended at the vessel walls of the posterior pituitary, suggesting axonal transport and secretion of VGF (Fig. 2e). However, VGF is not exclusively expressed in hypothalamic neurons. A subpopulation of VGF-immunoreactive epithelial cells was observed in the adenohypophysis (Fig. 2f).

To examine the relationship among VGF, schizophrenia and energy homeostasis, the association of BMI with hypothalamic VGF immunoreactivity was investigated. Despite the lack of direct correlations between BMI and VGF-immunopositive cell counts, the diagnosis-dependent reduction of VGF in schizophrenia subjects was particularly significant in patients with a BMI $\leq$28.7 kg/m^2. These results suggest that the observed effects are prominent in subjects without metabolic syndrome [34]. The cellular and metabolic conditions in patients with schizophrenia are dramatically different from those in VGF knockout mice, which are thin, small, hypermetabolic, hyperactive, and relatively infertile with markedly reduced leptin levels, fat stores, and secretion of VGF peptides [38]. Moreover, VGF mRNA levels are induced in the normal mouse

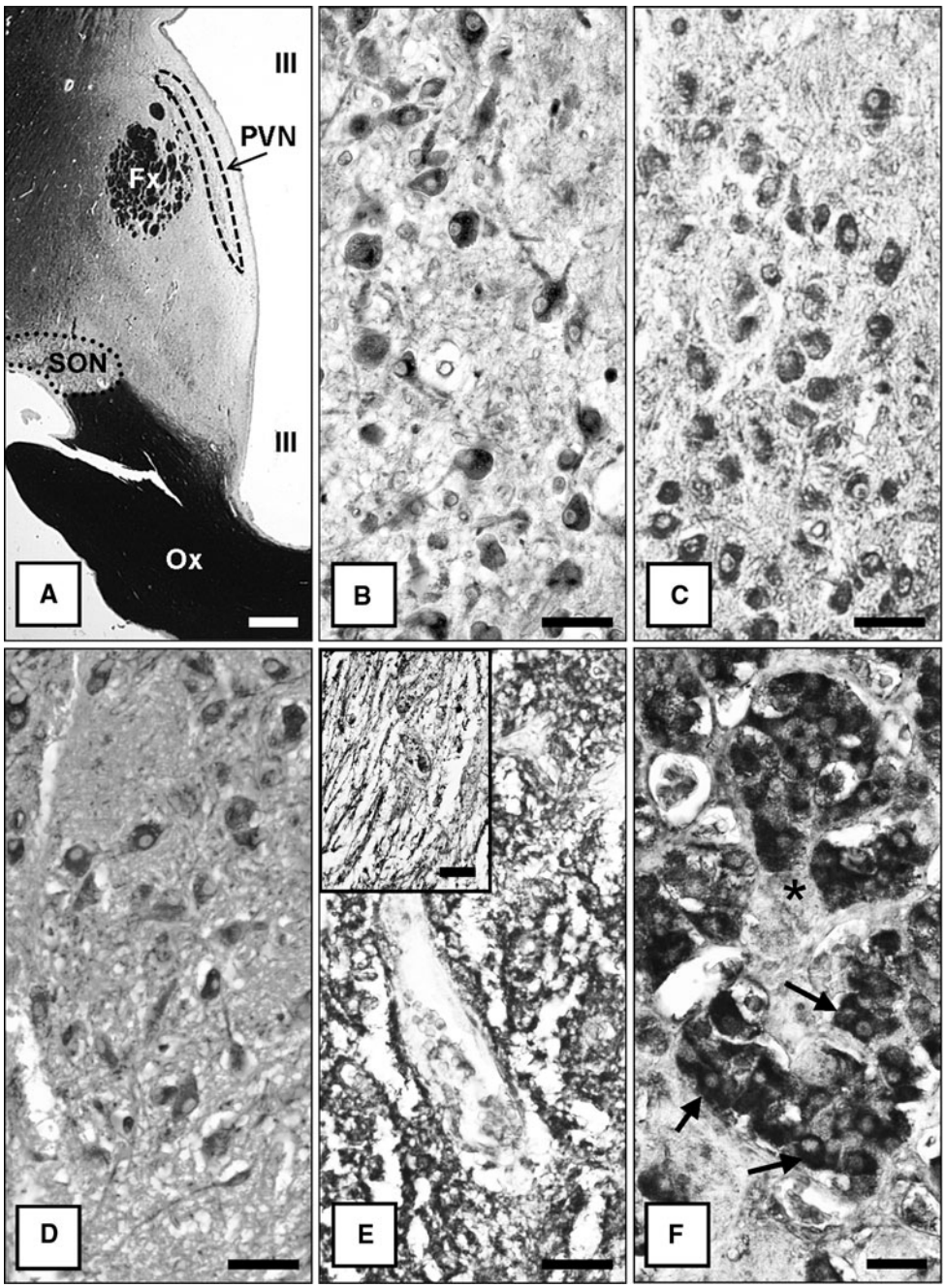

Fig. 2 **a** Low-power photomicrograph of a Nissl–myelin stained coronary section illustrating the delineation of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. **b** D20-VGF-immunopositive PVN and **c** SON neurons. **d** Comparative image of R15-VGF-immunopositive SON neurons, showing a slightly inferior signal-to noise ratio compared with D20 immunostains. **e** Posterior pituitary with D20-VGF-immunopositive fibers that ended at a vessel wall, suggesting axonal transport and secretion of VGF. The detailed image shows D20-VGF-stained axons from the pituitary stalk. **f** Anterior pituitary with D20-VGF-immunopositive (*arrows*) and D20-VGF-negative (*star*) epithelial cells. *Annotation:* III, third ventricle; *Fx* fornix, *Ox* optic chiasm. *Bars*: A = 1 mm; B, C, D, E, E and F = 50 μm

hypothalamic arcuate nuclei in response to fasting [15]. A previous study has reported that first-onset, drug-naïve schizophrenia patients exhibit increased glucose levels in CSF due to increased levels of VGF fragment 23-62 and decreased insulin sensitivity [17]. Another VGF-derived peptide, TLQP-21, affects catabolism in rodents [4]. Therefore, VGF is a key regulator of energy homeostasis and forms at least two metabolically active peptides with seemingly antagonistic properties, whereupon a complete loss of the VGF gene in mice causes a lean and hypermetabolic phenotype [4]. Further studies are needed in the future to evaluate different VGF cleavage peptides in the PVN and SON of patients with schizophrenia.

VGF may be a central factor in the pathophysiology of schizophrenia, linking hypotheses of decreased insulin and growth factor signaling with impaired energy homeostasis, synaptic function and neurogenesis [10]. Because the expression of VGF is induced by NGF, BDNF, neurotrophin 3, epidermal/fibroblast growth factors and insulin (see "Introduction"), schizophrenia-related disturbances in

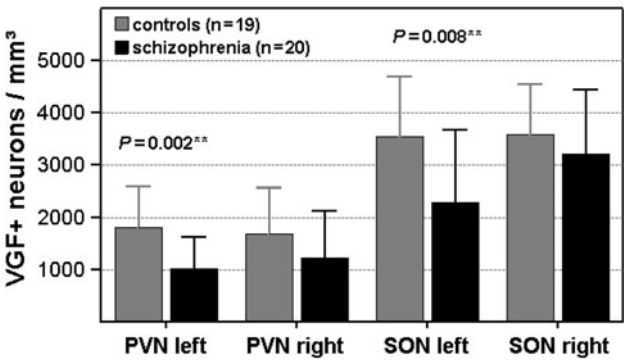

Fig. 3 Patients with schizophrenia showed significantly lower D20-VGF-immunopositive neuron densities in the *left* paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus. *Annotation:* Data are presented as mean ± standard deviation, ** $P < 0.01$

hypothalamic VGF expression may be a consequence of reduced insulin [13, 14, 33] or growth factor signaling [11, 12, 31, 37]. In addition, reduced secretion of VGF peptides in schizophrenia may impair synaptic functions [1, 9] and neurogenesis [24, 32].

Regarding other neuropsychiatric disorders, Cocco et al. [8] have shown reduced levels of several VGF peptides in the parietal cortex of alzheimer's disease (AD) samples and decreased levels of VGF in the CSF. However, Zhao et al. [40] have demonstrated reduced levels of VGF in the CSF of patients with amyotrophic lateral sclerosis (ALS). Ruetschi and colleagues [25] have described a decrease in VGF levels in the CSF of patients with frontotemporal dementia (FTD). Altogether, it has been suggested that VGF and its smaller processed peptides play a distinguishing role in many neurological and psychiatric diseases [3]. Ryan et al. [26] have observed a basal pituitary hyperactivity in first-episode, drug-naïve patients with schizophrenia, indicating an important role for the VGF gene in the synaptic plasticity of the HPA axis. Interestingly, a reduced brain volume has been detected in AD, FTD, and schizophrenia. Changes in VGF expression may promote neurodegeneration that may be due to a loss of synaptic plasticity. Postnatally, VGF gene expression gradually increases in the first 2 weeks, which is when synapses with definitive target neurons are established, and subsequently gradually decreases at the end of the plasticity period [35].

In the current study, correlation analyses suggest that the data were not confounded by other epidemiological factors (age and gender), although the activity of psychosis tends to be higher at the age of 20–30 years. Therefore, other factors seem to be responsible for the differential VGF expression. We excluded autolysis and fixation time as a reason for the observed differences. Even disease-specific factors (duration of disease and medication dosage) did not exhibit significant correlations with VGF expression.

The present study has certain limitations that need to be considered. First, because the amount of extracellular VGF was not quantified in our immunohistochemical study, we cannot directly assess whether reduced neuronal VGF immunoreactivity reflected increased VGF secretion. Second, mass spectrometry may allow a better determination of different VGF cleavage products which cannot be discriminated by immunohistochemistry. Third, although drug-naïve schizophrenia patients may exhibit symptoms of the metabolic syndrome [14], most atypical neuroleptics aggravate these symptoms. However, the influence of atypical neuroleptics remains unclear because all patients in the present study were medicated with typical antipsychotic drugs.

Summary and conclusion

Based on previous observations of neuroendocrine and hypothalamic deficits in schizophrenia, and to determine whether recently described increases of the VGF fragment 23-62 in CSF are related to changes in hypothalamic VGF expression, an immunohistochemical study was performed in 20 patients with schizophrenia and 19 matched control subjects.

VGF immunohistochemistry showed immunostained fibers in the pituitary stalk and neurohypophysis that ended at vessel walls, suggesting axonal transport and secretion of VGF. The cell density of VGF-immunoreactive neurons was reduced in the left PVN ($P = 0.002$) and SON ($P = 0.008$) of patients with schizophrenia.

This study provides the first evidence for diminished hypothalamic VGF levels in schizophrenia, which might suggest increased protein secretion. Our finding was particularly significant in patients with a BMI $\leq$28.7 kg/m^2. These results indicate that the observed effects are more prominent in subjects without metabolic syndrome. VGF may be a central factor in the pathophysiology of schizophrenia, linking hypotheses of decreased growth factor and insulin signaling with impaired synaptic function, neurogenesis, and energy homeostasis.

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Conflict of interest The authors declare that they have no conflict of interest.

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