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ORIGINAL COMMUNICATION

Norbert Galldiks
Alexander Thiel
Cathleen Haense
Gereon R. Fink
Rüdiger Hilker

^{11}C-Flumazenil positron emission tomography demonstrates reduction of both global and local cerebral benzodiazepine receptor binding in a patient with Stiff Person Syndrome

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Dr. N. Galldiks, MD (✉) · A. Thiel, MD · G. R. Fink, MD
Dept. of Neurology
University of Cologne
Kerpener Str. 62
50924 Cologne, Germany
Tel.: +49-221/478-4029
Fax: +49-221/478-6454
E-Mail: norbert.galldiks@uk-koeln.de

G. R. Fink, MD
Institut für Neurowissenschaften INB 3 – Kognitive Neurologie
Forschungszentrum Jülich

R. Hilker, MD
Dept. of Neurology
Goethe University, Frankfurt Main

C. Haense, MD
Max-Planck Institute for Neurological Research, Germany

Abstract Stiff Person Syndrome (SPS) is a rare autoimmune disorder associated with antibodies against glutamic acid decarboxylase (GAD-Ab), the key enzyme in γ-aminobutyric acid synthesis (GABA). In order to investigate the role of cerebral benzodiazepine-receptor binding in SPS, we performed [^{11}C]flumazenil (FMZ) positron emission tomography (PET) in a female patient with SPS compared to nine healthy controls. FMZ is a radioligand to the postsynaptic central benzodiazepine receptor which is co-localized with the GABA-A receptor. In the SPS patient, we found a global reduction of cortical FMZ binding. In addition, distinct local clusters of reduced radiotracer binding were observed. These data provide first in vivo evidence for a reduced postsynaptic GABA-A receptor availability which may reflect the loss of GABAergic neuronal inhibition in SPS.

Key words [^{11}C]flumazenil PET · GABA-A-receptor-associated protein · antibodies against glutamic acid decarboxylase · GABA-A receptor

Introduction

Recent evidence suggests that the Stiff Person Syndrome (SPS) is caused by an autoimmunologically mediated process [1]. In up to 50 % of cases, SPS occurs in association with other autoimmune diseases, such as Diabetes mellitus type 1, Grave's disease, Hashimoto-Thyreoiditis and pernicious anemia, or as a paraneoplastic syndrome [9]. In approximately 80 % of SPS patients, autoantibodies against glutamic acid decarboxylase (GAD-Ab) exist, suggesting an autoimmune-mediated impairment of γ-aminobutyric acid (GABA)ergic neuronal function as a key mechanism underlying muscle hyperactivity [10]. However, the pathogenetic role of GAD-Ab in SPS remains to be further elucidated. We present the case of a female patient with SPS and reduced global and local cortical and subcortical benzodiazepine receptor binding detected with [^{11}C]flumazenil (FMZ) positron emission tomography (PET). FMZ is a radioligand which binds to the central benzodiazepine receptor which is co-localized with the GABA-A receptor. FMZ binds to a subunit of the postsynaptic GABAergic complex and is a reliable marker of neuronal integrity [4].

Case presentation

A 53-year-old woman presented with increased rigidity of her legs, particularly after long sitting or standing times and after exposure to coldness with symptom onset about one year prior to admission. She had a history of musicogenic reflex epilepsy for 30 years, treated with phenytoine 300 mg daily, and no more than two seizures

per year. Additionally, she was treated for restless legs syndrome with 6 mg ropinirole at night and for hypothyreoidism with 150 µg levothyroxine per day. Her family history was unremarkable. On examination, she had pronounced muscular rigidity of both legs which increased dramatically after sitting or standing for a long time and subsequent to coldness. Her walking distance was reduced and her gait was unstable. Neurological examination was otherwise normal. Laboratory testing revealed markedly increased serum GAD-Ab (> 300 U/l), a decreased vitamin B12 level (106 mg/l), autoantibodies against parietal cells, decreased TSH (0.24 mU/l) and ferritin (12 µg/l) levels and an increased homocysteine level (23.3 µmol/l). Amphiphysin antibodies were absent. EMG showed simultaneous motor unit activity in agonist and antagonist lower limb muscles. EEG, somatosensory and motor evoked potentials, as well as cerebral magnetic resonance imaging (MRI) were normal (see Fig. 1). MRI of the spine revealed a herniated vertebral disc of the cervical segment 6/7 without relevant spinal cord compression. Diagnosis of SPS was made and a pulse of 500 mg intravenous methylprednisolone was administered for five days, subsequently reduced to an oral methylprednisolone maintenance dose of 20 mg daily [9]. In addition, the substitution of iron and vitamin B12 was initiated. Under immunomodulatory treatment, motor symptoms improved slowly. Symptomatic therapy with antispastic drugs or benzodiazepines was not necessary.

Methods

After obtaining written informed consent, the patient was investigated before initiation of the immunomodulatory treatment with FMZ-PET in resting state using an ECAT HRRT scanner (Siemens/CTI, Knoxville, TN, USA). All medication was discontinued 12 hours before scanning. The individual patient data were compared to those of 9 healthy volunteers (2 female and 7 male; mean age, 61.4 y; range, 29–74 y) without central nervous disease and without any medication with central nervous action who were examined either on the ECAT EXACT HR or on the ECAT EXACT HRRT scanner. 20 mCi (740 MBq) of FMZ were injected intravenously, and the accumulation of FMZ was recorded for 60 minutes by dynamic serial scanning.

To ensure comparability of the PET data, all images underwent the same processing steps including Gaussian smoothing (12 mm width) and spatial normalization by statistical parametric mapping (SPM2; Wellcome Department of Imaging Neuroscience, University College London, U.K.). To generate parametric images, a proportional scaling of cortical FMZ binding relative to white matter activity was performed [16]. The latter was measured using ellipsoid shaped regions of interest (ROI) placed in the right oval center in the same x-, y-, and z-direction after coregistration of all PET images. From the scaled images of all healthy volunteers an average and a standard deviation image was derived. This average image was subtracted from the image of the patient and the difference image was divided by the control's standard deviation image on voxel-by-voxel basis, thus, yielding a z-transformed image of the patient's FMZ-binding. This image is displayed in a discrete color scale with one interval representing 0.5 z-scores (see Fig. 1).

All voxels with z-scores below –2 were regarded as showing a FMZ tracer binding out of the normal range. The Talairach coordinates of maximum significant abnormal voxels with reduced FMZ tracer binding were identified and labeled with the respective Brodmann areas (BA) using the Talairach & Tournoux Atlas [19].

Results

After z-transformation, a global decrease of cerebral FMZ binding was found in the SPS patient (Fig. 1). The mean z-score was –0.56 ± 0.49 in a circular ROI including the whole brain at the level with the best representation of the basal ganglia. In addition, significant clusters of locally reduced FMZ binding with a z-score below –2 were found in the right primary motor cortex (BA 4),

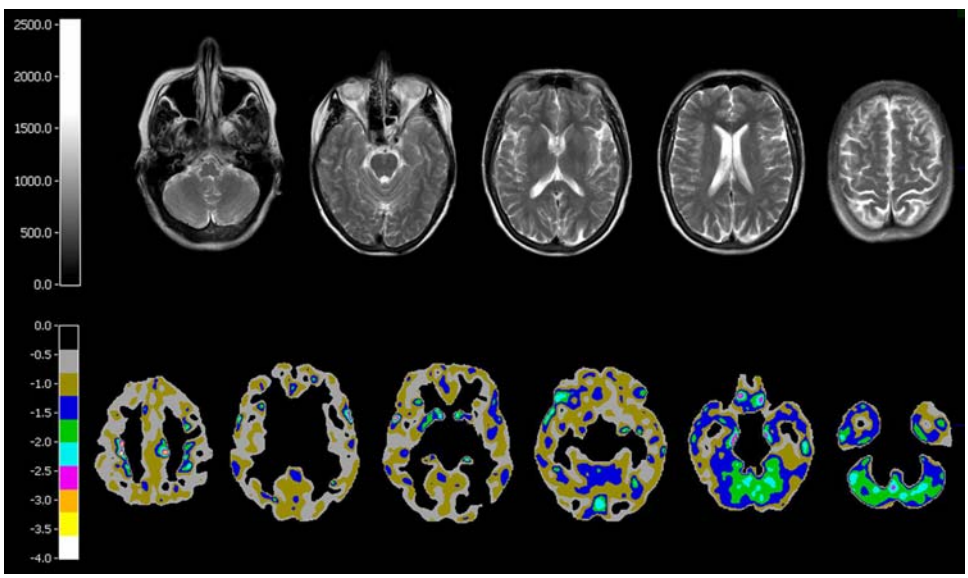

Fig. 1 Transaxial T2-weighted MR images of the SPS patient. No pathology was observed (upper panel). Transaxial PET z-maps of the FMZ binding of the SPS patient on voxel-by-voxel basis (lower panel). Voxels are shown for z-score thresholds ranging from 0 to –4 (by steps of 0.5; Bronson color scale). Significant changes were defined at a z-threshold below –2

right supplementary motor area (BA 6), right orbitofrontal cortex (BA 10, 11, 47), right middle occipital cortex (BA 19), right parahippocampal gyrus and left cingulate gyrus (BA 28, 31), left supramarginal area (BA 40) and in the right and left caudate head. Infratentorial areas with reduced FMZ binding were found in the cerebellar tonsils and in the vermis (Fig. 1, Table 1).

Discussion

The key PET finding in our SPS patient is a global reduction of cortical FMZ binding together with significant clusters of locally reduced tracer binding compared to a control group. Our data suggest that cerebral GABA-A receptor binding is impaired both globally and locally in SPS which might substantially contribute to the disinhibition of neuronal activity with subsequent muscular hyperactivity, rigidity and stiffness. Interestingly, the coincident startle type musicogenic epilepsy might also result from an impaired GABAergic neuronal inhibition as suggested by previous animal studies [3].

FMZ is a ligand which binds to the central benzodiazepine receptor which is co-localized with the GABA-A receptor and a subunit of the postsynaptic GABAergic complex [11]. FMZ has been shown to be a reliable marker of cortical neuronal loss in various brain disorders, such as epilepsy [18], Alzheimer's disease [12], acute vegetative state [15] and stroke [5]. However, cortical neuronal loss is not a feature of SPS in neuropathologic studies [11] and was also ruled out in our SPS patient by a normal brain MRI (Fig. 1). Therefore, the reduced FMZ binding is best explained by reduced GABA-A receptor density on neuronal cell surfaces resulting in a reduced number of binding sites for the radiotracer. Our finding is of special interest in the light of a recent study conducted by Raju and colleagues describing autoimmunity in SPS patients against the GABA-A-receptor-associated protein (GABARAP), which is responsible for the stability and surface expression of the GABA-A receptor [14]. The authors concluded that GABARAP acts as an autoantigen in SPS patients in which antibody-mediated blocking results in a decreased availability of central postsynaptic benzodiazepine receptors. Our FMZ-PET data are in line with this hypothesis and provide, therefore, in vivo evidence for the involvement of postsynaptic GABA-A receptor dysfunction in the pathophysiology of muscle hyperactivity in SPS patients.

Table 1 Voxels with reduced FMZ tracer binding

Brodmann Area	Localizations (Gray matter)	x-, y-, z-coordinates (Talairach)	z-score
4	right precentral gyrus	36, −17, 39	−3.86
6	right precentral gyrus	57, 5, 9	−3.67
9	right superior frontal gyrus	9, 61, 36	−2.78
10″	right medial frontal gyrus	12, 35, −14	−2.81
11	right inferior frontal gyrus	13, 35, −16	−2.65
19	right middle occipital gyrus	27, −80, 11	−2.45
28	right parahippocampal gyrus	20, −10, −21	−2.78
31	left cingulate gyrus	−9, −22, 40	−3.25
40	left supramarginal gyrus	−31, −44, 35	−2.95
47	right middle frontal gyrus	39, 35, −7	−2.25
–	right caudate head	9, 6, 6	−2.44
–	left caudate head	−8, 7, 6	−2.35
–	right cerebellar tonsil	5, −53, −38	−2.26
–	right vermis	7, −85, −18	−2.69
–	left cerebellar tonsil	−24, −59, −34	−2.16

One possible explanation for the global reduction of brain FMZ binding in our SPS patient might be a systemic autoimmune disease process with antibodies against GABARAP and a resulting ubiquitous reduction of neuronal GABA-A receptor surface expression. In addition, however, clusters with local FMZ binding reduction were found in motor and premotor cortices which are likely to be related to the development of motor symptoms in SPS. These findings are fully compatible with previous studies using MR spectroscopy: Levy and colleagues demonstrated decreased GABA levels in the sensorimotor, cingulate and occipital cortex [7]. Another PET study by Perani and colleagues also reported an extensively decreased FMZ binding bilaterally in the premotor cortex of two SPS patients versus controls [13]. Despite the fact that the reasons for a regional selectivity of GABA transmitter dysfunction in SPS are currently unknown, the authors proposed a selective involvement of premotor-motor cortices due to the high demand of modulator control and a finely tuned GABAergic transmission in these regions [6].

From a methodological point of view, hypothetical drug effects on our PET findings have to be considered. It has to be pointed out that an affection of GABA-A receptors due to drugs included in the medication of the patient (phenytoine, ropinirole and levothyroxine) has previously been described, but a significant effect related to the benzodiazepine receptor or a local receptor selectivity was not observed [2, 8, 17]. Moreover, there is no evidence available for long-term alterations of cerebral benzodiazepine receptor binding due to any of these drugs.

In conclusion, our FMZ-PET data provide in vivo evidence that postsynaptic benzodiazepine receptor dysfunction contributes to the reduced GABAergic neuronal inhibition in SPS. From a clinical point of view, a reduced availability of GABA receptors in motor-related areas might be a major pathophysiological determinant of muscular hyperactivity and stiffness in SPS. Therefore, FMZ-PET seems to be a suitable tool to investigate the pathogenetic role of cerebral GABA transmitter dysfunction in SPS.

References

1. Dalakas MC, Fujii M, Li M, McElroy B (2000) The clinical spectrum of anti-GAD antibody-positive patients with stiff-person syndrome. Neurology 55: 1531–1535
2. Eden RJ, Costall B, Domeney AM, Gerrard PA, Harvey CA, Kelly ME, Naylor RJ, Owen DA, Wright A (1991) Preclinical pharmacology of ropinirole (SK&F 101468-A) a novel dopamine D2 agonist. Pharmacol Biochem Behav 38:147–154
3. Faingold CL (2002) Role of GABA abnormalities in the inferior colliculus pathophysiology – audiogenic seizures. Hear Res 168:223–237
4. Heiss WD, Graf R, Fujita T, Ohta K, Bauer B, Lottgen J, Wienhard K (1997) Early detection of irreversibly damaged ischemic tissue by flumazenil positron emission tomography in cats. Stroke 28:2045–2051; discussion 2051–2042
5. Heiss WD, Kracht LW, Thiel A, Grond M, Pawlik G (2001) Penumbral probability thresholds of cortical flumazenil binding and blood flow predicting tissue outcome in patients with cerebral ischaemia. Brain 124:20–29
6. Levy LM, Hallett M (2002) Impaired brain GABA in focal dystonia. Ann Neurol 51:93–101
7. Levy LM, Levy-Reis I, Fujii M, Dalakas MC (2005) Brain gamma-aminobutyric acid changes in stiff-person syndrome. Arch Neurol 62:970–974
8. Lingamaneni R, Hemmings HC Jr (2003) Differential interaction of anaesthetics and antiepileptic drugs with neuronal Na+ channels, Ca2+ channels, and GABA(A) receptors. Br J Anaesth 90:199–211
9. Meinck HM (2000) Stiff-man syndrome from neurological perspective. Internist (Berl) 41:455–459
10. Meinck HM, Faber L, Morgenthaler N, Seissler J, Maile S, Butler M, Solimena M, DeCamilli P, Scherbaum WA (2001) Antibodies against glutamic acid decarboxylase: prevalence in neurological diseases. J Neurol Neurosurg Psychiatry 71:100–103
11. Meinck HM, Thompson PD (2002) Stiff man syndrome and related conditions. Mov Disord 17:853–866
12. Meyer M, Koeppe RA, Frey KA, Foster NL, Kuhl DE (1995) Positron emission tomography measures of benzodiazepine binding in Alzheimer's disease. Arch Neurol 52:314–317
13. Perani D, Garibotto V, Moresco RM, Ortelli P, Corbo M, Fazio F, Folli F (2007) PET evidence of central GABAergic changes in stiff-person syndrome. Mov Disord 22:1030–1033
14. Raju R, Rakocevic G, Chen Z, Hoehn G, Semino-Mora C, Shi W, Olsen R, Dalakas M (2006) Autoimmunity to GABAA-receptor-associated protein in stiff-person syndrome. Brain 129: 3270–3276
15. Rudolf J, Sobesky J, Ghaemi M, Heiss WD (2002) The correlation between cerebral glucose metabolism and benzodiazepine receptor density in the acute vegetative state. Eur J Neurol 9:671–677
16. Rudolf J, Sobesky J, Grond M, Heiss WD (2000) Identification by positron emission tomography of neuronal loss in acute vegetative state. Lancet 355: 115–116
17. Sandrini M, Vergoni AV, Bertolini A (1993) No modifications of GABAA and benzodiazepine receptors following experimental dysthyroidism in rats. Pharmacol Res 28:47–52
18. Szelies B, Sobesky J, Pawlik G, Mielke R, Bauer B, Herholz K, Heiss WD (2002) Impaired benzodiazepine receptor binding in peri-lesional cortex of patients with symptomatic epilepsies studied by [(11)C]-flumazenil PET. Eur J Neurol 9:137–142
19. Talairach J, Tournoux P (1988) Coplanar Stereotaxic Atlas of the Human Brain: 3-Dimensional Proportional System – an Approach to Cerebral Imaging. Thieme, New York