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NO synthase-positive striatal interneurons are decreased in schizophrenia

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Abstract The gaseous messenger NO has repeatedly been suggested to play a role in the pathogenesis of psychoses. Following a pilot study, we investigated whether the number of nitrinergic neurons in the putamen of patients suffering from schizophrenia, bipolar disorder or major depression is altered. Post-mortem striatum sections of 15 brains from patients with either disease were examined by NADPH-diaphorase staining, which selectively labels NO synthase-positive interneurons. Quantification of these cells revealed significantly lower numbers of NO synthase-containing neurons in the putamen of schizophrenic patients. Our results suggest that striatal nitrinergic interneurons are involved in the pathophysiology of at least some forms of schizophrenia, such as e.g. catatonic schizophrenia.
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1. Introduction

Nitric oxide (NO) is a gaseous neurotransmitter thought to be implicated in a variety of higher CNS functions. At a molecular level, NO acts as a second messenger of the NMDA receptor in the hippocampus, whereas in the cortex and the basal ganglia, nitrosylation or cGMP-mediated mechanisms are important (Snyder and Ferris, 2000). Furthermore, it nitrosylates monoamine transporters (Kiss and Vizi, 2001). Due to these interactions, NO is a promising candidate molecule in the pathogenesis of endogenous psychoses, both on the genetic (Reif et al., 2006) as well as on the histopathological level (Bernstein et al., 2005). In the human brain, NO is predominantly formed by neuronal NO-Synthase (NOS-I). Initial histochemical studies already indicated an involvement of NOS-I in schizophrenia: by means of the NOS-specific NADPH diaphorase staining (NADPHd), Akbarian and colleagues demonstrated maldistribution of NOS-positive cells in the frontal and temporal lobes (Akbarian et al., 1993a,b). Other regions in which dysfunction of nitrinergic neurotransmission was found include the brain stem (Karson et al., 1991), the vermis (Karson et al., 1996) and nuclear regions (Bernstein et al., 1998; Garcia-Rill et al., 1995). The striatum has also repeatedly been suggested in the pathogenesis of schizophrenic psychoses. Holt et al. (1999) reported that cholinergic striatal

Abbreviations: Bip, bipolar disorder; DMSO, dimethylsulphoxide; ic, internal capsule; MD, major depression; NAPDHd, nicotinamide adenine dinucleotide phosphate diaphorase; NC, nucleus caudatus; NMDA, *N*-methyl-D-aspartate; NO, nitric oxide; NOS, nitric oxide synthase; PMI, post-mortem interval; Pu, corpus putamen; SMRI, Stanley Medical Research Institute; Sz, schizophrenia.

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interneurons are decreased in schizophrenia, and we could previously show that striatal volume is increased in schizophrenia (Lauer and Beckmann, 1997) paralleled by an increased number of neurons in the nucleus caudatus/nucleus accumbens complex (Beckmann and Lauer, 1997).

The molecular basis for striatal pathology however still remains elusive. Hence, it seems to be worthwhile to search for pathological alterations of striatal NOS-positive neurons. To do so, we have established a morphological classification of nitrinergic striatal interneurons based on NADPHd histochemistry (Johannes et al., 2003). Using this classification system, we demonstrated in a pilot study that nitrinergic interneurons in the striatum of schizophrenic subjects are morphologically altered and decreased in number (Lauer et al., 2005). In the present study, we sought to verify our initial findings in a larger sample of schizophrenic patients as well as extending the investigation to affective psychoses, under the a priori hypothesis that NADPHd positive cells are reduced in number in schizophrenic patients as compared to controls. Furthermore, the effect of potentially intervening variables was investigated for by means of multiple regression analysis.

2. Experimental procedures

2.1. Subjects

Frozen unfixed sections of the human striatum were obtained from the Stanley Foundation Neuropathology Consortium (Torrey et al., 2000). The collection consisted of 7 slices per brain, collected from 15 subjects suffering from schizophrenia, 15 from bipolar disorder (Bip; 11 thereof featuring signs of psychosis), 15 from major depression (MD) without psychotic features and 15 controls. All groups were matched for age, sex, race, post-mortem interval (PMI) and hemispheric side. Demographic information and medical data including lifetime use of psychotropic medications, history of drug abuse and last prescribed medication were provided by the Stanley Medical Research Institute (SMRI). All experiments were performed blinded to the diagnosis. All experimental procedures were in accordance with the Declaration of Helsinki, and complied with the ethical guidelines of the University of Würzburg.

2.2. NADPH-diaphorase staining

For detection of NOS-I expressing cells in the striatum, seven sections of each brain were processed for NADPHd labelling (Johannes et al., 2003). After 7 min of fixation in 4% paraformaldehyde, slices were washed 3×5 min in Tris–HCl buffer (pH 7.4). Afterwards they were transferred into the staining solution containing DMSO (1.2%, Sigma, Germany), nitro blue tetrazolium (0.4 mg/ml, Sigma), NADPH (2 mg/ml, Sigma) and Triton-X (0.3%, Sigma), dissolved in Tris–HCl. Each slice was incubated overnight under light protection in 1 ml of the staining solution. Subsequently, the slices were washed, air-dried and finally covered with VitroClud (R. Langenbrinck, Germany). Artifacts due to shrinkage were avoided since no aggressive dehydration procedure by passages in ascending grades of alcohol was performed.

2.3. Quantification

Human brain sections were examined semi-quantitatively to compare between different diagnostic entities using a brightfield Leica microscope (DMRBE, Wetzlar, Germany). Microphotographs were taken with a digital camera mounted on the microscope. Cell counts in the putamen were performed in a systematic random manner (Fig. 1A, B); as all counted particles were of comparable size, no Abercrombie correction was applied. By combination of tube length, eyepiece (10×/25) and objective (10/0.30), a microscopic field of 1 mm^2 (equivalent to a sampling volume of 0.014 mm^3, thickness of slices 14 μm), was defined. While the first field to be counted was chosen randomly, further fields followed in a defined distance of 2 mm.

2.4. Statistics

The average number of NADPHd labelled cells is reported as means ± SEM NOS-positive cells/mm^2 putamen (equivalent to 0.014 mm^3). Multiple regression analyses were performed to assess whether NADPHd cell number is influenced by potentially confounding variables (age at death, gender, brain pH and weight, days of storage, post-mortem interval (PMI), and brain hemisphere; Table 1) in all subjects. As

Table 1 Demographic data on the investigated subjects

Diagnosis (*n* = 15 each)	Brain hemisphere (*n* right)	Age at death	pH	PMI (h)	Storage (days)	Onset of disease (age)	Disease duration (yrs)	Neuroleptic treatment (lifetime quantity of fluphenazine or equivalent; mg)	Never received APs (*n*)	Severity of alcohol use	Suicide (*n*)
Control	7	48 ± 10	6.2 ± 0.2	23 ± 9	338 ± 234	n.a	n.a	n.a.	15	1.0 ± 1.0	n.a.
Schizophrenia	6	44 ± 13	6.1 ± 0.2	33 ± 14	621 ± 233	23 ± 8	21 ± 11	52266 ± 62061	1	1.9 ± 1.7	4
Bipolar disorder	8	42 ± 12	6.1 ± 0.2	32 ± 16	620 ± 172	21 ± 8	20 ± 10	20826 ± 24015	3	2.8 ± 1.8	9
Major depression	6	46 ± 9	6.1 ± 0.2	24 ± 11	434 ± 290	34 ± 13	13 ± 11	n.a.	15	1.9 ± 2.0	7

Every diagnostic group comprised of 9 males and 6 females. Severity of alcohol abuse was graded qualitatively by the SMRI from 0 to 5. APs, antipsychotics; n.a., not applicable.

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