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Increased density of AKAP5-expressing neurons in the anterior cingulate cortex of subjects with bipolar disorder

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ABSTRACT

Brain anatomical abnormalities as well as cognitive and emotional processing deficits have been reported for the prefrontal cortex in bipolar disorder, which are in part attributable to cellular and laminar abnormalities in postsynaptic protein expression. A kinase anchoring protein (AKAP) 5/79 plays a key role in postsynaptic signalling of excitatory synapses. We aimed to reveal if the cellular expression of AKAP5/79 protein is altered in the anterior cingulate cortex and the dorsolateral prefrontal cortex in bipolar disorder. Ten subjects with bipolar disorder and ten control cases were investigated by use of immunohistochemical and morphometric techniques. Compared with controls in subjects with bipolar disorder, the numerical density of AKAP5-expressing neurons was significantly increased in the left ($p = 0.002$) and right ($p = 0.008$) anterior cingulate cortex. Layer-specific counting revealed that left side layers II ($p = 0.000$), III ($p = 0.001$) and V ($p = 0.005$) as well as right side layers III ($p = 0.007$), IV ($p = 0.007$) and V ($p = 0.004$) had significantly increased AKAP5-positive cell densities in bipolar disorder. In contrast, no statistically significant differences were found for the dorsolateral prefrontal cortex. However, we observed a more intense intraneuronal immunostaining in both prefrontal areas in bipolar disorder patients. Elevated cell numbers and increased intracellular expression of AKAP, together with the altered expression patterns of most intracellular interaction partners of this protein in bipolar disorder as known from the literature, might point to disease-related abnormalities of the AKAP-associated signalosome in prefrontal cortex neurons.

1. Introduction

The A-kinase anchor proteins (AKAPs) form a family of structurally diverse proteins, which all bind with high affinity to the regulatory subunit of protein kinase A. A prominent member of AKAPs family is AKAP5/79/150. There are species orthologue products of the encoding gene *akap5*, which differ in their molecular weight (bovine AKAP75, human AKAP79, and rodent AKAP150; Gorny et al., 2012). Functionally, AKAP5/79 is a scaffold and anchoring protein located in postsynaptic densities of excitatory synapses that recruits the cAMP-dependent protein kinase A (PKA) and protein phosphatase 2B (calcineurin) to membrane-associated guanylate kinase (MAGuK)-linked AMPA-type glutamate receptors, thus contributing to the control of receptor phosphorylation and synaptic plasticity (Wong and Scott, 2004; Robertson et al., 2009). In addition, AKAP79/150 may coordinate regulation of AMPA receptor activity with spine structure directly through MAGuK binding and membrane–cytoskeletal interactions (Scott, 2003). Moreover, AKAP79/150 binds to the MAGuKs PSD95/SAP90 and SAP97, which constitute a bridge between AKAP79/150-anchored proteins and the AMPA- and NMDA-type glutamate receptors (Colledge et al., 2000; Swayze et al., 2004), but also with beta-1-adrenergic receptors (Gardner et al., 2007) and beta-2-adrenergic receptors (Fraser et al., 2000). While AKAP5 is widely acknowledged to be central to normal excitatory synaptic functioning (as recently reviewed in Ting et al., 2012), very little is currently known about its possible involvement in brain pathologic

Abbreviations: ACC, Anterior Cingulate Cortex; AKAP, A-Kinase Anchor(ing) Protein; AMPA, α-Amino-3-hydroxy-5-Methyl-4-isoxazolepropionic Acid; BD, Bipolar Disorder; DLPFC, Dorsolateral Prefrontal Cortex; DSM, Diagnostic and Statistic Manual of Mental Disorders; f, female; GTPase, Guanosine Triphosphate Hydrolase; MAGuK, m; male, Membrane-Associated Guanylate Kinase; PKA, NMDA; *N*-Methyl-D-Aspartic Acid, Protein Kinase A; PKC, Protein Kinase C; PSD, Postsynaptic Density; SAP, Synapse-Associated Protein; TREK, Twik Related Potassium (K) Channel.

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processes, particularly in neurodegenerative and neuropsychiatric disorders (Chang et al., 2003; Sanderson and Dell'Acqua, 2011). Recently, we demonstrated that AKAP5 is involved in aggression and anger (Richter et al., 2011). In order to learn more about AKAP signalling in diseases with severe emotional dysregulation, we decided to investigate AKAP expression in post-mortem brains of individuals with bipolar disorder (BD). AKAP5 might play a role in the pathophysiology of BD for the following reasons: (1) The human *akap5* gene maps to 14q21–q24 on chromosome 14, a region which has repeatedly been linked to BD (Liu et al., 2003; McInnis et al., 2003; Seguardo et al., 2003; Craddock et al., 2005; Kealey et al., 2005; Cassidy et al., 2007; Kato, 2007 and others), (2) an increased copy number of the *akap5* gene has been found in some BD patients (Wilson et al., 2006), (3) chronic mild stress decreases AKAP5 in rat amygdala, while antidepressants reverse AKAP5 expression (Surget et al., 2009); and (4) most of the numerous cellular binding/interaction partners are altered in their expression or biological activity in BD (for details see Table 5). We have focused our efforts on two prefrontal cortical areas, the pregenual anterior cingulate cortex (ACC) and the dorsolateral prefrontal cortex (DLPFC), because these two brain regions have repeatedly been reported to play important roles in BD, especially with regard to the compromised neurocognitive ability and impaired socio-emotional behaviour of BD patients in addition to the core mood symptoms (for recent considerations, see Bora et al., 2010; Gamo and Arnsten, 2011; Van Rheenen and Rossell, 2012; Zhao et al., 2012).

2. Material and methods

2.1. Human brain samples

All brains were from the Magdeburg Brain Collection. Sampling of the human brain material and asservation was done in accordance with the Declaration of Helsinki (1984), German law and approval by the local Ethics commission.

Brains were collected from ten patients with bipolar disorder (BD) I (three women, seven men). The age range was 36–66 years (mean age 50.25 years). Of these, four died by suicide. Control brains were collected from five women and five men with an age range of 33–65 years (mean age: 56.2 years). All control subjects died of natural causes. They have been in a depressive state at the time of death. Demographic data of the control cases are summarized in Table 1.

All patients were matched for age, gender and post-mortem interval. The matching processes were done prior to all analyses. Information for clinical diagnosis was obtained by the study of clinical records and by structured interviews with people who either lived with, or had frequent contact with, the subjects before death. Structured interviews were carried out to collect information to determine the presence or absence of psychiatric disorders. Data on lifetime and current mental illness were gathered with a Lifetime version of a Schedule for Affective Disorders and Schizophrenia (Spitzer et al., 1978). The final diagnosis was compatible with the DSM-III-R (1987). In the same way, neuropsychiatric disorders were excluded in the control subjects. There was no current or lifetime psychoactive substance disorder (abuse or dependence according to DSM-III-R) in any of the subjects. All patients suffering from BD received long-term treatment with antidepressants. In addition, some of them received neuroleptic drugs. Most bipolar patients had lithium. For medication details see Table 3.

Qualitative neuropathological changes due to neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Pick's disease), tumours, inflammatory, vascular or traumatic processes were ruled out by an experienced neuropathologist (for demographical data see Table 1 and Table 2).

Table 1
Demographical data for control subjects.

Individuals without psychiatric disorder (Controls)	Age (years)	Gender	Cause of death	Duration of illness (years)	Postmortem delay (hours)
1	50	m	cardiac insufficiency	0	72
2	64	f	cardiac and circulatory failure	0	24
3	33	f	Acute respiratory insufficiency	0	72
4	50	f	cardiac and circulatory failure	0	72
5	56	m	cardiac and circulatory failure, pulmonary insufficiency	0	24
6	63	m	Rupture of the aorta	0	24
7	65	f	Heart failure, arteriosclerosis	0	24
8	64	f	sudden cardiac death	0	26
9	63	m	Pulmonary embolism	0	48
10	54	m	Pneumonia	0	24
Mean	56.2 ± 10.1				41.0 ± 22.6

Table 2
Demographical data for the subjects with BD.

Individuals with bipolar disorder	Age (years)	Gender	Cause of death	Duration of illness (years)	Postmortem delay (hours)
1	69	m	Suicide (pills)	26	48
2	69	m	Suicide (hanging)	18	24
3	47	m	Heart failure	9	24
4	46	m	Suicide pills)	13	4
5	42	m	Pneumonia	16	12
6	62	f	Heart failure	11	72
7	60	f	Suicide (Shooting)	10	24
8	39	m	Heart failure	2	24
9	59	f	Pulmonary embolism	24	72
10	36	m	Pulmonary insufficiency	14	56
Mean	50.25 ± 12.8				36.0 ± 22.6

Table 3
Psychopharmacological treatment.

BD case	AD	N (CE)	BDZ	CBZ	Li
1	Fluphenazine (dosage unknown)	0	7	0	0
2	Maprotriptyline (200)	0	2	0	280
3	Amitriptyline (225)	0	0	0	558
4	Amitriptyline (50)	20	3	0	565
5	Amitriptyline (16)	133	18	0	0
6	Amitriptyline (Dosage unknown)	95	18	0	0
7	Amitriptyline (112)	0	11	600	450
8	Fluphenazine (3)*	52	0	0	0
9	Maprotriptyline (150)	112	11	0	0
10	Amitriptyline (75)	0	0	10	740

AD antidepressants; BZD benzodiazepines; CBZ carbamazepine; CE chlopromazine equivalents; Li lithium; N neuroleptics. All medications are given as mean daily dose (mg/d) over the last 90 days prior to death (see Bielau et al., 2005). *no medication during the last 10 days before death.