Journal of Psychiatric Research 55 (2014) 101–109

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Reduced connexin 43 immunolabeling in the orbitofrontal cortex in alcohol dependence and depression

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ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form
18 March 2014
Accepted 4 April 2014

Keywords:
Alcoholism
Major depressive disorder
Prefrontal cortex
Postmortem
Immunohistochemistry
Gap junctions

ABSTRACT

Reduced density of glial cells and low levels of some astrocyte proteins have been described in the orbitofrontal cortex (OFC) in depression and alcoholism, two disorders often comorbid. These regressive changes may also involve the communication between astrocytes via gap junctions and hemichannels, which play important regulatory roles in neurotransmission. We determined levels and morphological immunostaining parameters of connexin 43 (Cx43), the main protein subunit of astrocyte gap junctions/hemichannels, in the OFC of subjects with depression, alcoholism or comorbid depression/alcoholism as compared to non-psychiatric subjects. Postmortem brain samples from 23 subjects with major depressive disorder (MDD), 16 with alcohol dependence, 13 with comorbid MDD and alcohol dependence, and 20 psychiatrically-normal comparison subjects were processed for western blots to determine Cx43 levels. Area fraction of Cx43 immunoreactivity, and density and average size of immunoreactive puncta were measured in histological sections. There was a significant, larger than 60 percent decrease in Cx43 level in the three psychiatric groups as compared to controls. Area fraction of immunoreactivity and immunoreactive punctum size were reduced in all psychiatric groups, but Cx43-immunoreactive puncta density was reduced only in alcohol-dependent subjects. Among psychiatric subjects, no difference in Cx43 levels or immunostaining was found between suicides and non-suicides. The present data suggest that dysfunction of the OFC is accompanied by reduction in the levels of gap junction protein Cx43 in depression and alcoholism, and reduction in density of Cx43 immunoreactive puncta only in alcoholism, pointing to altered gap junction or hemichannel-based communication in the pathophysiology of those disorders.

1. Objectives of the study and background

Depression and alcoholism are often co-morbid, the presence of one of these disorders increasing the risk for the other and its severity (Gilman and Abraham, 2001; Greenfield et al., 1998; Hasin and Grant, 2002; Kranzler et al., 1996; Regier et al., 1990). Despite clear differences in diagnostic criteria and pathology, both disorders also share impediments in decision-making, emotional control and adequate planning, all functions crucially involving the prefrontal cerebral cortex (PFC) (Diekhof et al., 2008; O'Doherty, 2011). These impediments are associated with the neurophysiological and cellular pathology in the PFC that must underpin behavioral and emotional pathology in both major depressive disorder (MDD) (Drevets, 2007; Kringelbach and Rolls, 2004; Price and Drevets, 2012; Rajkowska, 2000) and alcohol-dependence (Dao-Castellana et al., 1998; Flatscher-Bader and Wilce, 2008; Miguel-Hidalgo et al., 2002; Sullivan et al., 2000).

Even in the absence of gross morphological abnormalities, disturbances in glial cells, and not just in neurons, may crucially mediate neural dysfunction in psychiatric disorders (Banasr et al., 2008; Rajkowska and Miguel-Hidalgo, 2007). In both depression and alcoholism the number or packing density of glial cells, including astrocytes, and the expression of astrocyte markers are reduced in the dorsolateral and orbitofrontal cortices, both subdivisions of the PFC (Khundakar and Thomas, 2009; Miguel-Hidalgo, 2009). Nevertheless, alcoholism and major depression can differ in specific aspects of astrocyte involvement (Miguel-Hidalgo and Rajkowska, 2003; Miguel-Hidalgo et al., 2010), such as the levels of astrocytic glutamate transporters or glutamine synthetase (Miguel-Hidalgo et al., 2010).

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http://dx.doi.org/10.1016/j.jpsychires.2014.04.007
0022-3956/

Some of the neuronal support functions of astrocytes (Haydon and Carmignoto, 2006; Volterra and Meldolesi, 2005) strongly depend on astrocytes' ability to communicate with each other and with other cells through gap junctions and hemichannels (Cotrina and Nedergaard, 2012; Figiel et al., 2007; Giaume et al., 1997; Rouach et al., 2002), mainly composed of the protein connexin 43 (Cx43), with a smaller involvement of connexin 30 (Cx30). Thus, deficient expression or modifications of Cx43 could contribute to prefrontal physiopathology. Recently, we showed that alcohol preference and intake increased after gap junction blockade in the PFC of rats (Miguel-Hidalgo et al., 2009). In addition, in alcoholism, gap junction communication and Cx43 processing may be directly affected by ethanol exposure (Wentlandt et al., 2004). Likewise, PFC astrocyte pathology in depression may involve gap junction alterations. Recent studies show reduced levels of Cx43 and Cx30 mRNA in the PFC of subjects with psychiatric diagnoses who died by suicide (Ernst et al., 2011) and low Cx43 mRNA in the locus coeruleus in MDD (Bernard et al., 2011).

The goal of the present study was to ascertain whether major depression and alcoholism are correlated with significant variations in the levels and tissue distribution of Cx43 immunoreactivity in the orbitofrontal cortex (OFC). The OFC is a PFC subdivision heavily involved in the regulation of emotion and decision-making, which are dysfunctional in both alcoholism and major depression (Austin et al., 2001; Dom et al., 2005; Drevets, 2007; Volkow and Fowler, 2000). In addition, structural and functional neuroimaging studies have revealed significant abnormalities in the OFC in depression and alcoholism (Dom et al., 2005; Drevets, 2007; Schulte et al., 2010).

2. Materials and methods

The protocol for tissue collection was approved by the Institutional Review Boards of the University Hospitals of Cleveland and the University of Mississippi Medical Center. Written informed consent was obtained from legal next-of-kin for informant-based retrospective diagnostic interviews. Postmortem brain tissues were collected at autopsy at the Cuyahoga County Coroner's Office. Cases with evidence of neurological injury or disorder, prolonged agonal states or coma were excluded.

Retrospective diagnoses according to the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV) (APA, 1994) followed next-of–kin interviews using the Structured Clinical Interview for DSM Axis I Disorders modified for third-person reporting (First et al., 1995). Interview notes and clinical histories were reviewed independently by two licensed mental health clinicians to reach consensus diagnoses. Urine and blood collected at autopsy were examined by the coroner for presence of psychoactive substances.

The OFC was dissected from the locks of PFC, then it was frozen, and stored at −80 °C, and the postmortem interval (PMI; time between death and freezing of tissue) noted. In each subject, brain pH was determined from frozen tissue. This study included the following groups of subjects: comparison subjects without diagnosis of a psychiatric disorder (COMP, $n = 20$), subjects with alcohol-dependence diagnosis alone (ALC, $n = 16$), subjects with major depressive disorder (MDD) alone ($n = 23$), and subjects with both MDD and alcoholism (MDA, $n = 13$). The MDD group included 17 subjects with an antidepressant prescription within the last month of life, 6 subjects that had not received antidepressant medication within the last month of life, and 2 had medication for only 4 days before death. In the MDA group, 4 subjects had no antidepressant prescription. Responsiveness to medication was estimated before laboratory experiments, with only two cases estimated after experiments. Estimates were made according to clinical impressions from antemortem information and next-of-kin testimony. In the ALC group, ethanol was detected in the blood of 11 subjects, with eight at or over the legal limit (0.08 g/dL). In the MDA group, ethanol was detected in three subjects, all over the legal limit. Table 1 presents a summary of demographic and medical descriptors.

Brodmann's area 47 was identified in the left OFC (Uylings et al., 2010), and frozen 50 μm- and 20 μm-thick sections were collected for use in western blot-based protein measurements and immunohistochemistry, respectively.

2.1. Western blotting

Sections were sampled with a punch (0.5 mm diameter) that included all cortical layers but not white matter. These samples were collected in vials, stored at −80 °C and later homogenized in 0.01 M Tris–HCl containing 1% SDS, 2 mM EDTA, and protease inhibitor. The homogenate was centrifuged at 4 °C and 12,000 *g* for 30 min. Supernatant containing 25 μg of protein was applied in duplicate to wells in 10% Bis-Tris precast gels in the NuPAGE Bis-Tris Electrophoretic System (Invitrogen, Carlsbad, California). After electrophoresis, gels were transferred to PVDF membranes, later incubated with anti-Cx43 mouse monoclonal antibody (clone 2/Connexin-43, from BD Transduction Laboratories, cat. no. 610061) diluted 1:500, washed and incubated with an alkaline phosphatase-conjugated secondary antibody. This monoclonal antibody was developed against a peptide containing amino acids 252–270 of rat Cx43 and specifically binds Cx43 in tissue from humans and other vertebrate species (Asrih et al., 2012; Niger et al., 2010; Labovsky et al., 2010). Membrane chemiluminescent bands

Table 1
Summary of descriptive variables for the subject groups.

Descriptives/Group	Comparison	ALC	MDD (no alco.)	MDA (comorbid alco.)
AGE	49.20 ± 11.10	47.81 ± 8.56	50.00 ± 16.45	52.31 ± 14.43
GENDER	12M, 8F	13M, 3F	15M, 8F	9M, 4F
Causes of death	17CV, 1BP, 1PN, 1MT	6SUIC, 8CV, 1APX, 1EG	18SUIC, 1HK, 1GW, 3CV	6SUIC, 6CV, 1PTE
ETHNICITY	14C, 6AA	15C, 1AA	20C, 2AA, 1A	13C
PMI	22.063 ± 6.73	22.06 ± 10.18	24.08 ± 7.36	26.29 ± 6.54
pH	6.50 ± 0.33	6.70 ± 0.18	6.55 ± 0.21	6.62 ± 0.23
ONSET (depr)	N/A	N/A	38.65 ± 17.23	32.69 ± 12.13
DURATION (depr)	N/A	N/A	11.15 ± 14.82	14.21 ± 10.56
ONSET (alco)	N/A	22.12 ± 5.37	N/A	24.38 ± 9.92
DURATION (alco)	N/A	25.75 ± 10.57	N/A	23.67 ± 13.27

Abbreviations: AA = African American, A = East Asian, APX = asphyxia, ALC = Alcohol dependent subjects, alco = alcohol dependence, BP = bronchopneumonia, C = Caucasian, CV = cardiovascular disease, depr = major depression, EG = esophagitis, F = female, GW = gunshot wound, HK = hyperkalemia, M = male, N/A = not applicable, MDD = major depressive disorder, MT = multitrauma in car accident, PMI = postmortem interval (time from death to freezing of brain samples), PN = pancreatitis, PTE = pulmonary thromboembolism, SUIC = suicide. AGE, ONSET and DURATION are given in years, and PMI in hours. Numerical values for AGE, PMI, pH, ONSET and DURATION are given as mean ± standard deviation.