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FADD ADAPTOR AND PEA-15/ERK1/2 PARTNERS IN MAJOR DEPRESSION AND SCHIZOPHRENIA POSTMORTEM BRAINS: BASAL CONTENTS AND EFFECTS OF PSYCHOTROPIC TREATMENTS

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Abstract—Enhanced brain apoptosis (neurons and glia) may be involved in major depression (MD) and schizophrenia (SZ), mainly through the activation of the intrinsic (mitochondrial) apoptotic pathway. In the extrinsic death pathway, pro-apoptotic Fas-associated death domain (FADD) adaptor and its non-apoptotic p-Ser194 FADD form have critical roles interacting with other death regulators such as phosphoprotein enriched in astrocytes of 15 kDa (PEA-15) and extracellular signal-regulated kinase (ERK). The basal status of FADD (protein and messenger RNA (mRNA)) and the effects of psychotropic drugs (detected in blood/urine samples) were first assessed in postmortem prefrontal cortex of MD and SZ subjects (including a non-MD/SZ suicide group). In MD, p-FADD, but not total FADD (and mRNA), was increased (26%, *n* = 24; all MD subjects) as well as p-FADD/FADD ratio (a pro-survival marker) in antidepressant-free MD subjects (50%, *n* = 10). In contrast, cortical FADD (and mRNA), p-FADD, and p-FADD/FADD were not altered in SZ brains (*n* = 21) regardless of antipsychotic medications (except enhanced mRNA in treated subjects). Similar negative results were quantified in the non-MD/SZ suicide group. In MD, the regulation of multifunctional PEA-15 (i.e., p-Ser116 PEA-15 blocks pro-apoptotic FADD and PEA-15 prevents pro-survival ERK action) and the modulation of p-ERK1/2 were also investigated. Cortical p-PEA-15 was not changed whereas PEA-15 was increased mainly in antidepressant-treated subjects (16–20%). Interestingly, cortical p-ERK1/2/ERK1/2 ratio was reduced (33%) in antidepressant-free when compared to antidepressant-treated MD subjects. The neurochemical adaptations of brain FADD (increased p-FADD and pro-survival p-FADD/FADD ratio), as well as its interaction with PEA-15, could play a major role to counteract the known activation of the mitochondrial apoptotic pathway in MD.

Key words: FADD adaptor, PEA-15, MAP kinases, major depression, schizophrenia, psychotropic drugs.

INTRODUCTION

Major depression (MD) and schizophrenia (SZ) are multifactorial major psychiatric syndromes whose pathogenic mechanisms remain largely unknown (Lewis and González-Burgos, 2008; Kupfer et al., 2012; Sibille and French, 2013; Howes and Murray, 2014). It has been postulated that aberrant increases in apoptotic cell death in the brain (neurons and glia) might contribute to the pathophysiology of MD and SZ. Thus, numerous postmortem and neuroimaging studies have revealed volume loss, atrophy and/or structural abnormalities in MD (e.g. Rajkowska et al., 1999; Cotter et al., 2001; Sheline, 2003; McKernan et al., 2009; Arnone et al., 2012; Oh et al., 2012; Grieve et al., 2013) and SZ (e.g. Benes et al., 1991, 2001; Wong and Van Tol, 2003; Honea et al., 2005; Arango et al., 2012), findings which are compatible with enhanced apoptosis in several brain regions including the prefrontal cortex (Jarskog et al., 2004; Lucassen et al., 2004; Glantz et al., 2006; Harlan et al., 2006; Catts and Weickert, 2012; Sellmann et al., 2014).

In this context, the main focus in MD and SZ postmortem brains has been the apoptotic intrinsic pathway (Galluzzi et al., 2009) with the demonstration of mitochondrial dysfunctions (e.g. increased pro-apoptotic Bcl-2-associated X protein (Bax)/B-cell lymphoma 2 (Bcl-2) ratio; Jarskog et al., 2004; Glantz et al., 2006), which can lead to oxidative damage to brain cells (Andreazza et al., 2010, 2013; but see Benes et al., 2003). In contrast to mitochondrial apoptosis, little is known on the status of the extrinsic cell death pathway

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Abbreviations: Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; ERK, extracellular signal-regulated kinase; FADD, Fas-associated protein with death domain; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MAPK, mitogen-activated protein kinase; MD, major depression; MEK, mitogen-activated protein kinase kinase (ERK kinase); mRNA, messenger RNA; p, phosphorylated; PARP-1, poly-(ADP-ribose)-polymerase-1; PEA-15, phosphoprotein enriched in astrocytes of 15 kDa; PFC/BA9, prefrontal cortex/Brodmann's area 9; PMI, postmortem interval; RNI, RNA integrity numbers; RT-qPCR, real-time quantitative polymerase chain reaction; SEM, standard error of the mean; SZ, schizophrenia.

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(Algeciras-Schimnich et al., 2002) in brains of MD or SZ subjects (Fas receptor messenger RNA (mRNA) expression was reported unchanged in SZ prefrontal cortex; Catts and Weickert, 2012). In this pathway, Fas-associated death domain (FADD) adaptor (Chinnaiyan et al., 1995; Ramos-Miguel et al., 2012) is a crucial step linking Fas receptor with the activation of initiator and executioner caspases with the final cleavage of vital cell substrates (Sastry and Rao, 2000; Kumar, 2007; Burke, 2008). FADD protein can also induce non-apoptotic actions including cell growth and differentiation (Park et al., 2005; Valmiki and Ramos, 2009; Galluzzi et al., 2012). Importantly, the phosphorylation (p) of FADD (p-Ser194 FADD in the human brain) is also essential for the non-apoptotic effects (including neuroplasticity) of this multifunctional protein (Alappat et al., 2005; García-Fuster et al., 2008a; Ramos-Miguel et al., 2009, 2010, 2012). A major regulator of FADD is a small molecule called phosphoprotein enriched in astrocytes of 15 kDa (PEA-15), which is also present in mature neurons (Araujo et al., 1993; Sharif et al., 2004; Ramos, 2008). Notably, p-Ser116 PEA-15 has been shown to prevent the pro-apoptotic function of FADD (Trencia et al., 2003; Renganathan et al., 2005) and non-p PEA-15 can block the nuclear effects of extracellular signal–regulated kinase (ERK1/2) (Formstecher et al., 2001; Sulzmaier et al., 2012; Mace et al., 2013), a member of the mitogen-activated protein kinase (MAPK) family (Roskoski, 2012). In previous studies, the activity of pro-survival ERK pathway (including Raf, mitogen-activated protein kinase kinase (ERK kinase) (MEK) or ERK kinase, and p-ERK1/2) was markedly reduced in brains of MD subjects (Dwivedi et al., 2001, 2006, 2009). In contrast, p-ERK1/2 activity was reported unchanged in SZ brains (Kyosseva et al., 1999; Yuan et al., 2010; Funk et al., 2012).

Against this background, the present study investigated the basal status of pro-apoptotic FADD (protein content and mRNA expression), anti-apoptotic p-Ser194 FADD, and associated partners PEA-15 and ERK1/2 (and other MAPK components) in postmortem brains of well-defined cohorts of subjects with MD or SZ (including a non-MD/SZ suicide group), as well as the effects of previous psychotropic medications. A preliminary account of this study was presented at the 11th World Congress of Biological Psychiatry (García-Sevilla et al., 2013).

EXPERIMENTAL PROCEDURES

Specimens of human prefrontal cortex

Samples of the right (Hecht, 2010) dorsal prefrontal cortex (PFC; Brodmann's area 9, BA9) from subjects with MD and SZ as well as from healthy matched controls were obtained at the time of autopsy in compliance with legal and ethical standard procedures (Rivero et al., 2013). The study was approved by the Ethics Committee of Clinical Investigation (CEIC-CAIB) and developed following the guidelines of the University of the Balearic Islands (UIB). The selected MD and SZ subjects (DSM-IV clinical diagnosis) had been used in previous postmortem neurochemical studies (Rivero et al., 2013). After quantitative toxicology (antidepressants, antipsychotics, other psychotropic drugs, and ethanol), subjects with MD or SZ [psychotropic-free: MD(−) or SZ(−); psychotropic-treated: MD(+) or SZ(+)] were matched to controls (negative toxicology) for postmortem interval (PMI), brain pH, gender and age at death (Table 1, which also includes the causes of death and blood/urine levels of psychotropic drugs in MD(+) and SZ(+) subjects). Because suicide was the main cause of death in MD and SZ subjects, a non-MD/SZ suicide group ($n = 9$; 5M/4F; PMI: 22 ± 3 h; age: 44 ± 4 yr) was also investigated in parallel (main diagnoses in this group: adjustment disorder and personality disorder). The influence of PMI on target proteins (total FADD and PEA-15) in the PFC/BA9 has been assessed (García-Fuster et al., 2008b; Ramos-Miguel et al., 2009). Because the p-active forms of ERK1/2 enzymes, but not those of p-FADD and p-PEA-15, are very sensitive (rapid degradation) to the effect of PMI (Ferrer-Alcón et al., 2004; Ramos-Miguel et al., 2009), p-ERK1/2 and other MAPK components were quantified in independent series of PFC/BA9 samples from MD (6F/8M) and control (6F/6M) subjects with shorter PMIs [all MD: PMI 12 ± 1.5 h; age 41 ± 4 yr, $n = 14$; MD(−): PMI 10 ± 1.6 h; age 41 ± 5 yr, $n = 7$; MD(+): PMI 14 ± 2.3 h; age 41 ± 6 yr, $n = 7$; controls: PMI 19 ± 2.9 h; age 43 ± 4 yr, $n = 12$)].

Quantification of target proteins by immunoblot analysis

The contents of FADD, PEA-15 and MAPKs in the PFC/BA9 of MD, SZ, and controls were assessed by standard gel electrophoresis (proteins were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by semi-quantitative Western blot analysis as described (García-Fuster et al., 2008b; Ramos-Miguel et al., 2009). The primary rabbit polyclonal antibodies (epitope-affinity purified) or monoclonal antibodies (dilution range: 1/1000 to 1/5000) were: anti-FADD (immunogen: peptide corresponding to amino acids 28–208 of human FADD; batches K1407 and D0109; Cat No. sc-5559; Santa Cruz Biotechnology, Santa Cruz, CA, USA); anti-p-FADD (immunogen: peptide containing p-Ser194 of human p-FADD; batches CO72 and H1610; Cat No. sc-12439; Santa Cruz Biotechnology); anti-PEA-15 (immunogen: peptide containing Leu60 of human PEA-15; batch 1; Cat No. 2780; Cell Signaling Technology, Danvers, MA, USA); anti-p-PEA-15 (immunogen: peptide containing p-Ser116 of human PEA-15; batch 0100B; Cat No. 44-836G; Invitrogen, Frederick, MD, USA); anti-Ras (immunogen: a synthetic peptide of human Ras; clone 18; Cat No. R02120; BD Transduction Laboratories, Lexington, KY, USA); anti-c-Raf-1 (immunogen: a synthetic peptide of human c-Raf-1; clone 53; Cat No. R19120; BD Transduction Laboratories); anti-MEK1/2 (immunogen: a synthetic peptide of human MEK1/2; batch 6; Cat No. 9122; Cell Signaling); anti-ERK1/2 (immunogen: a synthetic peptide of human p42 MAPK, batch D33075, Cat No. 442704, Calbiochem-Novabiochem, FRG); anti-p-ERK1/2 (immunogen: a peptide containing p-Thr202/p-Tyr204 of human p44 MAPK, Cat No. 9101,