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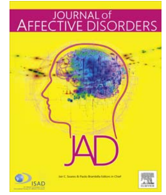

Research paper

Dysregulation of IRAS/nischarin and other potential I_1-imidazoline receptors in major depression postmortem brain: Downregulation of basal contents by antidepressant drug treatments

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ABSTRACT

Background: Major depressive disorder (MDD) has been associated with altered brain densities of imidazoline receptors (I_1-IR and I_2-IR types).
Methods: The contents of potential I_1-IR IRAS/nischarin (167 kDa) and, for comparison, those of I_1- (85 kDa) and I_2- (45 kDa and 30 kDa) IR proteins were quantified by western blotting in postmortem prefrontal cortex (PFC/BA9) of antidepressant-free ([MDD(−)], n=9) and antidepressant-treated ([MDD(+)], n=12) subjects and matched controls (n=19).
Results: In MDD, regardless of antidepressant treatment (n=21), IRAS/nischarin was not altered in PFC/BA9. However, the content of IRAS/nischarin was found modestly and not significantly increased (+19%, p=0.075) in MDD(−) and significantly decreased (−24%, p=0.001) in MDD(+), revealing that basal I_1-IR content was downregulated by antidepressants. Putative 85 kDa I_1-IR was upregulated (+35%, p=0.035) in MDD(−) but it was not reduced (−14%, p=0.37) in MDD(+). There was a positive correlation (r=0.33, p=0.037, n=40) between the contents of IRAS/nischarin and 85 kDa IR proteins in PFC/BA9 (control and MDD subjects). In MDD and regardless of antidepressants, the content of cortical 45 kDa I_2-IR was increased (+31%, p=0.006) and that of 30 kDa I_2-IR decreased (−14%, p=0.002), indicating basal dysregulations of these potential IRs.
Limitations: MDD(+) subjects had been treated with a variety of antidepressant drugs. The results must be understood in the context of suicide victims with MDD.
Conclusions: The dysregulation of IRAS/nischarin in depressed brains is a major novel finding that supports a role of this potential I_1-IR in the neurobiology of MDD and in the molecular mechanisms of antidepressant drugs.

1. Introduction

The existence of specific receptors for compounds bearing an imidazoline moiety was suggested various decades ago (Bousquet et al., 1984) and to date three main types of imidazoline receptors (IR) have been described. Even though their molecular nature remains to be established, I_1-IRs, mediating sympathoinhibitory actions of drugs such as clonidine or moxonidine (Ernsberger and Haxhiu, 1997), and I_2-IRs, associated with neuroprotection and analgesia (Olmos et al., 1999; Li and Zhang, 2011), are widely expressed in brain and peripheral tissues, whereas I_3-IRs, responsible for insulin secretagogue activity of certain imidazolines, are found in pancreatic islets (Chan et al., 1994). Further, several substances with properties of an endogenous IR ligand have been proposed, including agmatine, the decarboxylated form of arginine (Li et al., 1994).

Over the past decades various studies have suggested the involvement of IR proteins (I_1- and/or I_2-IR types) and the putative endogenous ligand agmatine (and its degrading enzyme agmatinase) in the neurobiology of major depressive disorder (MDD), with a possible therapeutic usefulness of specific IR ligands (e.g. see García-Sevilla et al., 1996a, 1996b; Halaris et al., 2002; Halaris and Piletz, 2003; Piletz et al., 2008). Thus, I_1-radioligand binding sites, labeled with [^{3}H]- or [^{125}I]p-clonidine, were found increased in platelets and brains of unmedicated MDD subjects (Piletz et al., 1996a) and antidepressant treatment was associated with I_1-IR normalisation (Piletz et al., 1996b; Zhu et al., 1999; Halaris et al., 2002), suggesting the platelet I_1-IR as a state marker of depressive symptomatology (Piletz et al., 2008). In turn, reduced densities of I_2-radioligand ([^{3}H]-

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idazoxan) binding sites were reported in platelets of depressed patients (Piletz et al., 1994) and in prefrontal cortex (PFC) of suicide victims (Sastre and García-Sevilla, 1997). In addition, certain selective I_2-ligands, associated with the induction of antidepressant effects, reduced the activity of monoamine oxidases in the brain, which were shown to possess I_2-binding sites (Nutt et al., 1995; Tesson et al., 1995).

Interestingly, the altered densities of IRs (radioligand binding sites) in MDD correlated with the contents of immunoreactive IR proteins (molecular masses of ≤85 kDa) using the anti-imidazoline receptor binding protein (IRBP) polyclonal antibody (Wang et al., 1993; García-Sevilla et al., 1999; Halaris and Piletz, 2003). More recently, the cloning of I_1-IR candidate proteins (about 167 kDa), namely human imidazoline receptor antisera selected (IRAS; identified with the anti-IRBP antibody; Piletz et al., 2000a) and its mouse homolog nischarin (Alahari et al., 2000), has been reported, which could represent functional IRs, precursors of IR entities of lower molecular masses (e.g. 85 kDa, detected with anti-IRBP and anti-IRAS antibodies, Piletz et al., 2000a) or subunits of complex structures responsible for binding of imidazoline compounds (discussed in Halaris and Piletz, 2003; Keller and García-Sevilla, 2015).

In contrast to classic IR proteins (≤85 kDa), full-length 167 kDa IRAS/nischarin has not been measured in brains of depressed patients. Therefore, the present study quantified the immunocontent of the I_1-IR candidate IRAS/nischarin and, for comparison, the densities of other immunoreactive IR proteins (potential $I_{1/2}$-IR types) in postmortem prefrontal cortex of unmedicated and antidepressant-treated MDD subjects and healthy controls. The major finding demonstrates the participation of IRAS/nischarin in the neurobiology of MDD and in the actions of antidepressant drugs.

2. Experimental procedures

2.1. Postmortem human brain samples

Samples of the right dorsal prefrontal cortex (PFC; Brodmann's area 9, BA9) from healthy controls and subjects with antemortem clinical diagnosis of MDD (DSM-IV) were obtained at the time of autopsy (Institutes of Forensic Medicine, Geneva, Switzerland, and Bilbao, Spain). The study was developed in compliance with the respective national policies of research and ethical review boards for human postmortem brain studies. The PFC/BA9 was selected for examination because it is one of the most consistently implicated brain regions in the pathophysiology of MDD, which in addition expresses an abundance of IRs (e.g. García-Sevilla et al., 1996a). The selected controls and MDD subjects had been used in previous postmortem neurochemical studies dealing with the regulation of some brain markers in MDD (Rivero et al., 2013; García-Fuster et al., 2014). The criteria for the selection of control subjects were absence of neuropsychiatric disorder or drug abuse (medical information), accidental cause of death, and negative toxicology for psychotropic drugs, except benzodiazepines (see Rivero et al., 2013 for further details for controls and MDD subjects). Suicide was the main cause of death in subjects with MDD and although suicide may have some specific neurobiology (Desmyter et al., 2011; Dwivedi and Pandey, 2011; Pandey, 2013), altered contents of IR proteins did no appear to contribute to suicide independently of MDD (for other brain markers see Rivero et al., 2013; García-Fuster et al., 2014). After quantitative toxicology (antidepressants, other psychotropic drugs, ethanol), subjects with MDD [antidepressant-free: MDD(−); antidepressant-treated: MDD(+)] were matched to healthy controls (with negative toxicology) for postmortem interval (PMI), brain pH, gender and age at death (Table 1, including causes of death and the various antidepressant drugs detected in blood/urine of MDD(+) subjects). A few control (one) and MDD (two) subjects were positive for benzodiazepines (oxazepam, midazolam) and ethanol (seven subjects) in blood and/or urine samples, but a possible influence of these agents on IR content in brain was discarded (García-Sevilla et al., 1996a). Brain pH is a better indicator of postmortem human brain tissue quality (e.g. for protein degradation) than the assessment of RNA integrity numbers (RIN) (Sonntag et al., 2016). The contents of neuron-specific enolase (NSE-2), a marker of neuronal damage (Nogami et al., 1998), were quantified and found very similar in brains of healthy control and MDD subjects (Table 1). Values of brain pH and NSE-2 indicated good PFC/BA9 tissue quality. The present 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).

Table 1
Demographic variables, postmortem interval (PMI), brain pH, and content of neuron-specific enolase-2 (NSE-2) of healthy controls and major depressive disorder (MDD) subjects.

Groups and subgroups	Gender (F/M)	Age (yr)	PMI (h)	Brain pH	NSE-2 (% C)
Healthy controls(C, n=19)	11/8	53 ± 4	23 ± 2	6.46 ± 0.04	100 ± 5
Major depressivedisorder (MDD, n=21)	12/9	56 ± 4	19 ± 2	6.39 ± 0.05	111 ± 7
Antidepressant-free[MDD(−), n=9]	6/3	64 ± 5	24 ± 4		112 ± 9
Antidepressant-treated[MDD(+), n=12]	6/6	50 ± 4	16 ± 2		110 ± 10

F:female, M:male, yr:years, h:hours. Healthy controls mainly died of accidental causes (heart attack, traffic trauma, drowning, homicide) and had a negative toxicology in blood and/or urine samples. Suicide (hanging, jumping, gun shot, drug overdose) was the main cause of death in MDD subjects. MDD(+) [antidepressant drugs detected in blood and/or urine]: citalopram, duloxetine, fluoxetine, mirtazapine, venlafaxine.

2.2. Sample preparation, immunoblot assays and quantification of target proteins

Brain samples (~100 mg) were prepared for immunodetection of IR proteins as described elsewhere (e.g. García-Fuster et al., 2014; Keller and García-Sevilla, 2015). All tissue preparations contained a mixture of six protease inhibitors (AEBSF, aprotinin, leupeptin, bestatin, pepstatin A, E-64; Protease Inhibitor Cocktail, P-8340, Sigma-Aldrich, St Louis, MO) and various phosphatase inhibitors (Phosphatase Inhibitor Cocktail 3, P-0044, Sigma-Aldrich). The bicinchoninic acid (BCA) assay (Pierce Biotechnology) was used for determination of sample protein concentration. Brain proteins (40 µg) were resolved by SDS-PAGE on 10% polyacrylamide minigels (Bio-Rad, Hercules, CA) followed by standard Western blot procedures (Keller and García-Sevilla, 2015). The nitrocellulose membranes were incubated overnight with the following specific primary antibodies (Ab):anti-NISCH Ab, #ab56849, lot:GR111152 (Abcam, Cambridge, UK) (see 2.3. for other features); anti-IRBP antiserum, lot:2 (Wang et al., 1993) (see 2.3. for other features); anti-enolase-2 Ab, #H9536, lot:1 (Cell Signaling, Danvers, MA**)**; anti-β-actin Ab, #A1978, lot: 065M4837V (Sigma-Aldrich). Horseradish peroxidase-conjugated secondary antibodies (anti-rabbit, anti-mouse, #7074 and #7076, resp., Cell Signaling) and ECL detection system (Amersham, Buckinghamshire, UK) were used to visualize immunoreactivity of target proteins on autoradiographic films (Amersham ECL Hyperfilm). The immunoreactive bands were quantified (integrated optical density, IOD) by densitometric scanning (GS-800 densitometer, Bio-Rad). The amount of a target protein in brain samples from MDD subjects was compared with that of matched controls (100%) in the same gel, and normalized to the contents of β-actin. Each brain sample was quantified in 2–4 gels and the mean value was used as a final estimate.

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