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Expression of PSA-NCAM and synaptic proteins in the amygdala of psychiatric disorder patients

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ABSTRACT

Neuroimaging has revealed structural abnormalities in the amygdala of different psychiatric disorders. The polysialylated neural cell adhesion molecule (PSA-NCAM), a molecule related to neuronal structural plasticity, which expression is altered in schizophrenia, major depression and in animal models of these disorders, may participate in these changes. However, PSA-NCAM has not been studied in the human amygdala. To know whether its expression and that of presynaptic markers, was affected in psychiatric disorders, we have analyzed post-mortem sections from the Stanley Neuropathology Consortium, which includes controls, schizophrenia, bipolar and major depression patients. PSA-NCAM was expressed in neuronal somata and neuropil puncta, many of which corresponded to interneurons. Depressed patients showed decreases in PSA-NCAM expression in the basolateral and basomedial amygdala; synaptophysin and GAD67 were also decreased, while VGLUT-1 was increased, in different nuclei. Increases in PSA-NCAM expression were found in the lateral nucleus of bipolar patients; synaptophysin and GAD67 were reduced, and VGLUT-1 increased, in their basolateral and lateral nuclei. The expression of synaptophysin and GAD67 was downregulated in the basolateral nucleus of schizophrenics. These results indicate that inhibitory and excitatory amygdaloid circuits are affected in these disorders and that abnormal PSA-NCAM expression in depressive and bipolar patients may underlie these alterations.

1. Introduction

Psychiatric disorders, such as major depression, schizophrenia or bipolar disorder are considerably different from many perspectives. However, all these disorders share abnormalities in the structure of the amygdala (reviewed in Drevets et al., 2008; Sheline et al., 1998; Tebartz van Elst et al., 2000). These structural changes may reflect alterations in neuronal structure, leading to disturbances in the balance of excitatory and inhibitory neurotransmission, which may lead to the functional abnormalities observed in patients. Chronic stress, which is considered a precipitating factor for these disorders and is accepted as a depression model (McEwen, 2000), induces dendritic atrophy and reductions in spine density in principal neurons of the hippocampus (Magarinos and McEwen, 1995; Sousa et al., 2000) and the medial prefrontal cortex (mPFC) (Radley et al., 2004, 2006). However, it has the opposite effect in certain amygdaloid nuclei, where it promotes dendritic outgrowth and increases spine density (Vyas et al., 2002, 2003). However, structural changes are not limited to principal neurons, they also affect inhibitory networks. A recent study from our laboratory has shown that mice subjected to chronic stress undergo dendritic hypertrophy in interneurons of the basolateral and lateral amygdala (Gilabert-Juan et al., 2011). This finding is in agreement with previous studies, which indicate that alterations in GABAergic neurotransmission probably play a crucial role in major depression, schizophrenia and bipolar disorder. Neurodevelopmental defects may lead to the appearance of a defective GABA system, particularly under stressful conditions. It has been postulated that the amygdala, especially its basolateral nucleus, may contribute to these abnormalities through an increased flow of excitatory activity (Benes and Berretta, 2001; Phillips, 2003). Interestingly, stress or high levels of corticosteroids also induce activation of the amygdala, leading to an increase in the excitability of principal neurons (Duvarci and Pare, 2007; Roozendaal et al., 2009). This increase in excitability can be also the result of a stress-induced reduction in

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inhibitory neurotransmission, which has also been reported in the amygdala (see Davis et al., 1994) for review.

The amygdaloid complex plays a critical role in the recognition and the response to emotional stimuli, including fear and anxiety (Adolphs et al., 1995; Cahill and McGaugh, 1998; Davis et al., 1994; LeDoux, 2000), which are abnormal in subjects suffering from these disorders. In addition, the amygdala is intimately connected to regions, such as the frontal lobe, which are involved in many psychiatric disorders, thereby influencing the storage and processing of these stimuli in cortical areas (Amaral and Insausti, 1992; McDonald, 1998). Consequently, structural alterations in amygdaloid regions may lead to dysfunction of other key neuronal systems implicated in affective and behavioral regulation. In fact, several studies have reported changes in the volume of the amygdala in psychiatric patients, which probably reflect structural plasticity of amygdaloid neurons (reviewed in Drevets et al., 2008; Sheline et al., 1998; Tebartz van Elst et al., 2000).

Structural changes in neurons are likely to be mediated by the expression of cytoskeletal proteins and cell adhesion molecules. In this regard, one of the molecules that has received more attention in recent years is the polysialylated form of the neural cell adhesion molecule (PSA-NCAM). NCAM can incorporate long chains of polysialic acid, which confer it anti-adhesive properties and, consequently, allow neurons to participate in plastic events, such as neurite outgrowth (Zhang et al., 1992) or synaptic reorganization (Seki and Rutishauser, 1998). In adult mammals, this molecule is expressed in cerebral regions where neuronal structural plasticity has been described, some of which are also involved in the pathogenesis of mental disorders: such as the mPFC (Varea et al., 2005, 2007a) or the hippocampus (Seki and Arai, 1991, 1993; Ni Dhuill et al., 1999). The study of different cortical regions of adult rats (Gilabert-Juan et al., 2011; Varea et al., 2005) and humans (Varea et al., 2007c) has shown that most PSA-NCAM expression is associated to interneuronal structures, suggesting that this molecule is involved in the plasticity of inhibitory networks. Abundant PSA-NCAM expression has also been described in the amygdala of adult rats (Nacher et al., 2002a) and mice (Nacher et al., 2010), where it is also expressed by interneurons (Gilabert-Juan et al., 2011). The presence of PSA-NCAM immunoreactive cells has also been described, with less detail, in non-human primates (Bernier et al., 2002). However, there is no data on the PSA-NCAM expression pattern or on the neurochemical phenotype of the elements expressing this molecule in the amygdala of adult humans.

Studies in adult rats have shown that changes in the levels of PSA-NCAM expression occur in parallel to the structural remodeling of neurons in the amygdala, mPFC and the hippocampus after chronic stress or chronic antidepressant treatment. Particularly in the amygdala, PSA-NCAM expression is downregulated after chronic stress (Cordero et al., 2005), as well as after chronic treatment with the antidepressant fluoxetine (Varea et al., 2007b), and these decreases are accompanied by parallel reductions in the expression of synaptic markers (Varea et al., 2007b). Moreover, some lines of evidence involve amygdaloid NCAM and PSA-NCAM expression with the synaptic plasticity associated with emotional learning: Mice with a targeted disruption of the NCAM gene display strong deficits in an amygdala-dependent learning task, the auditory fear conditioning (Stork et al., 2000), and this same task induces an increment in PSA-NCAM expression in the amygdala of rats (Markram et al., 2007).

Given its involvement in neuronal structural plasticity (Gascon et al., 2007; Rutishauser, 2008; Sandi, 2004), its abundant expression in the rodent amygdala (Cordero et al., 2005; Nacher et al., 2002a, 2010) and its modulation by aversive experiences and antidepressant treatment (Cordero et al., 2005; Guirado et al., 2009; Varea et al., 2007a,b), PSA-NCAM may mediate the structural changes observed in the amygdala of patients suffering from different mental disorders, particularly those affecting inhibitory neuronal networks. Moreover, as it has been observed in rodents (Castillo-Gomez et al., 2008; Varea et al., 2007b), changes in PSA-NCAM expression may occur in parallel to those in synaptic proteins and may indicate the presence of synaptic remodeling. It is specially interesting to investigate the impact of major depression, schizophrenia and bipolar disorder on the expression of proteins related to inhibitory networks, since several lines of evidence indicate that GABAergic neurotransmission plays an important role in these psychiatric disorders. Consequently, using immunohistochemistry, we have analyzed the expression of PSA-NCAM and that of synaptophysin (SYN), vesicular glutamate transporter type 1 (VGLUT-1) and glutamic acid decarboxylase-67 (GAD67), markers of generic, excitatory and inhibitory synapses respectively, in different amygdaloid nuclei from post-mortem samples of the Stanley Foundation Neuropathology Consortium, which includes tissue from control, schizophrenia, major depression and bipolar disorder patients. In order to study the neurochemical phenotype of neuropil elements and somata expressing PSA-NCAM in the human amygdala, we have also performed fluorescence immunohistochemistry and confocal analysis using antibodies against synaptophysin, glutamic acid decarboxylase (GAD67), Ca^2+/calmodulin dependent protein kinase II (CAMKII) a marker of excitatory neurons, and NeuN a marker of mature neurons.

2. Material and methods

2.1. Samples and histological processing

Frozen, 14 μm thick coronal sections containing the basolateral complex of the caudal amygdala were obtained from the Stanley Foundation Neuropathology Consortium. Five adjacent sections were obtained from similar rostrocaudal levels of the amygdala from 60 individuals divided into four groups ($n = 15$): normal control subjects and patients with bipolar disorder, major depression without psychotic features and schizophrenia. Diagnoses were made according to Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria. Group demographic summaries are described in Table 1. All brains underwent clinical neuropathological examination by two neuropathologists, none demonstrated evidence of neurodegenerative changes or other pathological lesions.

Sections were thawed and immediately fixed by immersion in a solution of paraformaldehyde 2.5% in a lysine-phosphate buffer, pH 7.4 for 20 min at room temperature. The lysine-phosphate buffer was prepared 1:1 from a solution of phosphate buffer 0.1 M pH 7.4 (PB) and a solution 0.2 M of lysine adjusted to pH 7.4 using a solution of Na_2HPO_4 0.1 M. The buffer was mixed with a concentrated solution of paraformaldehyde 3:1 and 0.214 g of sodium peryodate was added for each 100 ml just before use. After fixation, sections were washed in PB and processed immediately for immunohistochemistry. All the sections studied passed through the procedures simultaneously, to minimize any difference from histochemical and immunohistochemical protocols themselves.

Additional sections from human control amygdala were obtained from frozen samples from the Neurological Tissues Bank of the University of Barcelona and were used for the characterization of the neurochemical phenotype of PSA-NCAM expressing somata and puncta. Samples were obtained from five subjects without any neurological abnormality, the average age was 59.6 years (42–74) and the average time post-mortem before freezing the samples was 8.5 h (3.5 to 17.5). The tissue was thawed and fixed by immersion in the same fixative solution described above. After fixation, samples were washed using PB and 50 μm sections were obtained using a vibratome. Sections were then postfixed in the same solution for