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Research report

Serotonin 2A receptor clustering in peripheral lymphocytes is altered in major depression and may be a biomarker of therapeutic efficacy

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ABSTRACT

Background: In a previous report, we showed that the clustering of serotonin (5HT) transporter (SERT) protein on cell membranes of peripheral lymphocytes predicts responsivity to antidepressant medication in two subpopulations of naïve depression patients (Rivera-Baltanas et al., J Affect Disord, 2012, 137, 46–55). In this study, we extended this idea to 5-HT2A receptor clusters in a similar patient population.
Methods: We collected blood samples from a subset of patients from our previous study on SERT clustering (20 untreated and newly diagnosed depression patients, and 20 matched control subjects). Blood samples were collected at the time of diagnosis and after 8 weeks of pharmacological treatment and at analogous times in control subjects. We used the Hamilton scale to quantify the level of depression in patients both before and after treatment. We then used immunocytochemistry to assess 5-HT2A receptor clusters in lymphocytes at the same time points.
Results: We found that both the size and number of 5-HT2A receptor clusters were increased in naïve depression patients compared to control subjects. Interestingly, there were individual differences in the distribution of 5-HT2A receptor cluster size that allowed us to differentiate the depression patients into two subgroups: a D-I group and a D-II group. After 8 weeks of pharmacological treatment, patients in both groups showed an improvement of symptoms, but patients in the D-II group had a much better outcome with many of them showing remission of symptoms. Furthermore, although treatment decreased cluster number and size in both D-I and D-II groups, only the D-II patients showed an increase in the number of clusters within the modal peak. Importantly, the same patients that belonged in the D-I or D-II groups in the present report were also assigned to the same groups in our previous study on SERT clustering.
Limitations: The data should be replicated within a proper clinical trial.
Conclusions: 5-HT2A receptor clusters in peripheral lymphocytes are altered in major depression, partially reversed by antidepressant treatment, and may be considered a putative biomarker of therapeutic efficacy in major depression.

1. Introduction

Major depression is a severe and devastating neuropsychiatric illness that affects roughly 16% of the population (Kessler et al., 2003). Individuals suffering from depression have increased physical illness, decreased social functioning, and a high mortality rate, resulting in huge social and economic strain (reviewed in Belmaker and Agam, 2008). Although numerous antidepressants have been developed, up to one third of patients are non-responders to most currently used medications (Rush et al., 2006; Wijeratne and Sachdev, 2008). This response rate underscores the importance of developing novel biomarkers of therapeutic efficacy for depression, which would ultimately increase the potential for personalized medical treatment for these patients (reviewed in Rivera-Baltanas and Caruncho, 2010).

In a recent report we demonstrated that alterations in clustering of the serotonin transporter (SERT) along the membrane of

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peripheral blood lymphocytes could be used to differentiate naïve depression patients from controls. Importantly, the pattern of SERT clustering also identified two subpopulations of naïve depression patients [i.e., Depression I (D-I) and Depression II (D-II)] that responded very differently to 8 weeks of antidepressant treatment despite having similar Hamilton Depression Rating Scale (HDRS) scores before treatment. Specifically, the D-I patients had more SERT clusters per lymphocyte but these clusters were smaller in size than those seen in the D-II patients. Within the D-I group, 45% of patients were non-responders, 33% were responders without remission, and 22% showed symptoms of remission after treatment. However, in the D-II group, all patients were responders and 75% of them showed symptoms of remission (Rivera-Baltanas et al., 2012). Therefore, it is possible that the pattern of SERT protein clustering within peripheral lymphocytes could identify which patients are most likely to respond to a standard course of antidepressant treatment upon their initial diagnosis.

The serotonergic system is a complex one, and in addition to the SERT, the serotonin 2A receptor (5-HT2A) is also highly expressed in peripheral blood lymphocytes (Stefulj et al., 2000; Ahern, 2011; Inoue et al., 2011). Binding studies have shown that although peripheral SERT is downregulated in depression (see Mossner et al., 2007), the B-max of 5-HT2A receptors is increased in depression in both peripheral tissues and the central nervous system (Pandey et al., 1990, 1995). These increases in 5HT2A receptors are partially reversed by antidepressant treatment (Van Oekelen et al., 2001, 2003; Hanley and Hensler, 2002) suggesting that they could play some role in the neurobiology of depression. In addition, 5-HT2A receptors, like other G-protein coupled receptors (or transporters like SERT), tend to cluster in specific membrane microdomains, where they interact with and are regulated by resident proteins (Bhatnagar et al., 2004; Allen et al., 2007, 2008; Bjork et al., 2010). The functional importance of 5-HT2A receptor clustering, as well as the different alterations observed in binding studies of SERT and 5-HT2A receptors in depression, prompted us to wonder whether 5-HT2A receptor clustering in lymphocytes from patients with depression could be altered in a different manner than what we previously observed with SERT clustering. Therefore, we examined 5-HT2A receptor clusters in lymphocyte samples from the same population of patients used in our previous study. We did it this way so that we could compare in each individual patient the data that we obtained for 5-HT2A receptor clustering, with that previously obtained for SERT clustering (see Rivera-Baltanas et al., 2012). Knowledge about the pattern of 5-HT2A receptor clustering could shed additional light on the two subpopulations of naïve depression patients revealed in our previous study and help to determine whether peripheral membrane protein clustering could serve as a general biomarker for treatment responsivity in depression.

2. Methods

2.1. Subjects

The subjects in this experiment were a subset of patients and controls that we previously studied in our analysis of SERT clustering (Rivera-Baltanas et al., 2012). We had a group of naïve patients with a new diagnosis of major depression ($n=20$) and a control group with no history of mental illness ($n=20$). The criteria for inclusion, as well as the use of the Hamilton Depression rating Scale (HDRS) were as explained in Rivera-Baltanas et al. (2012). All participants gave their written informed consent with the research protocol being approved by the University of Santiago de Compostela ethics committee. We collected blood samples from depression and completed the HDRS test both at the time of recruitment, and after 8 weeks of psychopharmacological medication. Blood samples from matched control subjects were obtained from the blood bank of the University Hospital at Santiago de Compostela. For more detail on these procedures see Rivera-Baltanas et al. (2012).

Table 1
Demographic and clinical caracterization of study subjects.

	Control	Depression	Statistics
Number	20	20	
Age	35,14 ± 1,13	43,4 ± 2,42	$t(18) = -1{,}28$ $p(0{,}28)$
Gender			
Men	11 (55%)	8 (40%)	$\chi^2(1) = 1{,}07$ $p(0{,}21)$
Women	9 (45%)	12 (60%)	

Demographic data from control and depressed patients are reported in Table 1. Pharmacological treatments for each patient are provided in Supplementary Table 1.

2.2. Isolation of Blood lymphocytes and immunocytochemistry

The collection of blood samples and isolation of lymphocytes was as described in Rivera-Baltanas et al. (2012). After isolation of lymphocytes the samples were fixed for1 minute in a solution of 1% paraformaldehyde in phosphate buffer at room temperature, lymphocytes were then processed for immunocytochemistry using a specific antibody for the 5-HT2A receptor.

Immunolabelling of 5-HT2A receptors was performed by consecutive centrifugation and re-suspension of the lymphocyte samples in every step (see Rivera-Baltanas et al., 2010; Rivera-Baltanas et al., 2012). Lymphocytes were incubated for 10 min at 4 °C in a solution of 100 ml of human IgG (Sigma), diluted in PBS with 1% BSA, to block membrane immunoglobulins. This was followed by incubation for 12 h at 4 °C with anti-5-HT2A primary antibody (rabbit anti-5-HT2A polyclonal antibody, RA24288, NEUROMICS) diluted 1:100 in PBS with 1% BSA. This antibody has been previously characterized in several studies (e.g., Deltheil et al., 2008; Magalhaes et al., 2010; Yadav et al., 2011), and we also ascertained the specificity of the antibody by western blot following the protocol described in Giannaccinni et al. (2010) (see Fig. 1A). Samples were further incubated for 1 h at room temp with goat anti rabbit secondary antibody conjugated with Alexa Fluor 488 (Molecular Probes, A11008) diluted 1:200 in PBS with 1% BSA. After repeated washing, the samples were extended onto slides, coverslipped, and mounted with Moviol medium (Calbiochem). Finally, the samples were maintained at −20 °C until analysis by confocal microscopy. Omission of the primary antibody resulted in a complete absence of labelling.

Lymphocyte samples were studied and photomicrographs obtained in a laser confocal microscope (Leica TCS-SP2). Images of 5-HT2A receptor clusters in 100 lymphocytes per sample were analyzed using Image-J 1.42 imaging software (NIH), which automatically quantified the number of 5-HT2A receptor clusters per lymphocyte and also measured the size of those clusters (see Fig. 1C–F). This procedure was similar to that used in our previous study on SERT clustering (Rivera-Baltanas et al., 2012).

2.3. Statistical analyses

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, v 16.0, Chicago, USA). As we did for SERT clustering (Rivera-Baltanas et al., 2012), we examined the statistical significance of group differences in a number of ways. First, we examined group differences in demographic (age and gender) and clinical characteristics (diagnosis) using a two-tailed *t*-test for age and Chi-square tests for gender and diagnosis. Second, we examined group differences in HDRS scores measured