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Hippocampal transcriptional and neurogenic changes evoked by combination yohimbine and imipramine treatment

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ABSTRACT

Adjunct α_2-adrenoceptor antagonism is a potential strategy to accelerate the behavioral effects of antidepressants. Co-administration of the α_2-adrenoceptor antagonist yohimbine hastens the behavioral and neurogenic effects of the antidepressant imipramine. We examined the transcriptional targets of short duration (7 days), combination treatment of yohimbine and imipramine (Y + I) within the adult rat hippocampus. Using microarray and qPCR analysis we observed functional enrichment of genes involved in intracellular signaling cascades, plasma membrane, cellular metal ion homeostasis, multicellular stress responses and neuropeptide signaling pathways in the Y + I transcriptome. We noted reduced expression of the α_{2A}-adrenoceptor (*Adra2a*), serotonin $5HT_{2C}$ receptor (*Htr2c*) and the somatostatin receptor 1 (*Sstr1*), which modulate antidepressant action. Further, we noted a regulation of signaling pathway genes like inositol monophosphatase 2 (*Impa2*), iodothyronine deiodinase 3 (*Dio3*), regulator of G-protein signaling 4 (*Rgs4*), alkaline ceramidase 2 (*Acer2*), doublecortin-like kinase 2 (*Dclk2*), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (*Nfkbia*) and serum/glucocorticoid-regulated kinase 1 (*Sgk1*), several of which are implicated in the pathophysiology of mood disorders. Comparative analysis revealed an overlap in the hippocampal regulation of *Acer2*, *Nfkbia*, *Sgk1* and *Impa2* between Y + I treatment, the fast-acting electroconvulsive seizure (ECS) paradigm, and the slow-onset chronic (21 days) imipramine treatment. Further, Y + I treatment enhanced the quiescent neural progenitor pool in the hippocampal neurogenic niche similar to ECS, and distinct from chronic imipramine treatment. Taken together, our results provide insight into the molecular and cellular targets of short duration Y + I treatment, and identify potential leads for the development of rapid-action antidepressants.

1. Introduction

α_2-adrenoceptors are considered putative targets for rapid-action antidepressants (Blier, 2003). Several studies have indicated enhanced α_2-adrenoceptor expression, binding and functional responses within limbic brain regions, including the hippocampus, in both patients with major depressive disorder and animal models of depression (Escribá et al., 2004; Flügge et al., 2003; Rivero et al., 2014; Valdizán et al., 2010). Chronic antidepressant treatments evoke a downregulation or desensitization of the α_2-adrenoreceptor, a process suggested to influence the duration of therapeutic lag associated with pharmacological antidepressants (Invernizzi and Garattini, 2004; Subhash et al., 2003). Clinical studies indicate that antidepressants such as mirtazapine, which exhibit α_2-adrenoceptor antagonism, exert faster behavioral effects when administered alone or in conjunction with a classical antidepressant (Carpenter et al., 2002; Quitkin et al., 2001). The α_2-adrenoceptor antagonist yohimbine when co-administered with the antidepressant fluoxetine hastens antidepressant responses in patients with major depressive disorder (Sanacora et al., 2004), as well as in animal models (Dhir and Kulkarni, 2007). Preclinical studies (Yanpallewar et al., 2010) also indicate that combination treatment with the α_2-adrenoceptor antagonist yohimbine and the tricyclic antidepressant, imipramine (Y + I) elicits neurogenic, neurotrophic and behavioral changes within 7 days, as compared to imipramine treatment which requires 3 weeks of treatment to evoke similar responses. These studies suggest that α_2-adrenoceptor antagonism is a putative target for stand-alone or combination antidepressant therapy.

Currently, the molecular and cellular consequences of treatments such as the combination Y + I treatment, that bring together α_2-adrenoceptor antagonism with a classical pharmacological antidepressant treatment, remain poorly elucidated. To gain an understanding of the global gene expression changes that accompany the rapid

Abbreviations: ECS, electroconvulsive seizure; GFP, Green Fluorescent Protein; RNA, Ribonucleic Acid; PCR, Polymerase chain reaction; PFA, paraformaldehyd; PB, phosphate buffer; GFAP, glial fibrillary acidic protein; QNP, quiescent neural precursor; SGZ, subgranular zone; GCL, granule cell layer; DG, dentate gyrus

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behavioral outcomes of the Y + I treatment paradigm, we carried out microarray studies to assess the transcriptional targets of Y + I treatment within the hippocampus, a brain region that is known to be a target of antidepressant therapy. Further, we also evaluated the specific progenitor stage within the hippocampal neurogenic niche that is targeted by Y + I treatment, as hippocampal neurogenesis has been strongly linked to the behavioral effects of antidepressants. Finally, we addressed whether the transcriptional and neurogenic targets of the short duration Y + I treatment are also influenced by distinct antidepressant treatments such as the fast-acting electroconvulsive seizure (ECS) paradigm and the long duration (21 days) treatment with the classical antidepressant imipramine (I) with a view to identifying common molecular signatures across diverse antidepressant therapeutic strategies.

2. Methods

2.1. Animals

Adult male Wistar rats (250–400 g, 2–3 months of age) and adult male nestin-GFP transgenic mice (25–35 g, 2–3 months of age) generated as previously described (Yu et al., 2005) were group housed. Animals were maintained on a 12 hour light/dark cycle with access to food and water *ad libitum*. Nestin-GFP reporter mice were a kind gift from Dr. Steven Kernie (Columbia University, New York, USA) (Yu et al., 2005). Nestin-GFP mice were maintained on a C57BL/6J background and express green fluorescent protein (GFP) under the control of the nestin promoter, thus facilitating the identification of distinct stages of neural precursor development. All animal procedures were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the TIFR Institutional Animal Ethics committee.

2.2. Animal treatments

2.2.1. Drug treatments

To assess the global transcriptional changes evoked within the hippocampus by the fast-acting antidepressant combination of α_2-adrenoceptor blockade with yohimbine, along with the classical antidepressant imipramine, animals received yohimbine (2 mg/kg; Sigma, St Louis, MO, USA) 30 min prior to administration of imipramine (20 mg/kg; Sigma) (Y + I group) or received vehicle treatment (Ctl group), once a day for seven consecutive days. Two independent Y + I treatment groups were processed for microarray (n = 3 per group) and qPCR analysis (n = 9–10 per group). To determine hippocampal gene expression changes elicited by chronic antidepressant treatment, rats received imipramine (I, 20 mg/kg) or vehicle treatment (Ctl) once daily for 21 days, and were sacrificed 2 h following the final treatment (n = 8–10 per group). To assess the hippocampal transcriptional changes evoked by 7 days of imipramine treatment, rats received imipramine (20 mg/kg) or vehicle treatment (Ctl) once daily for 7 days, and were sacrificed 2 h following the final treatment (n = 10–11 per group). To examine the effects of Y + I treatment on quiescent neural precursors (QNPs) in the hippocampal neurogenic niche, nestin-GFP reporter mice received Y + I or vehicle treatment (n = 5–6 per group) as described above and were sacrificed 24 h after the last injection. To assess the effects of 21 day imipramine treatment on the QNP pool, nestin-GFP mice were treated with imipramine (20 mg/kg) or vehicle once daily for 21 days and sacrificed 24 h after the last injection (n = 5–6 per group). The vehicle for all drugs was 0.9% saline, except for yohimbine in which case the vehicle was 10% DMSO. The choice of drug doses was based on previous studies (Sairanen et al., 2005; Yanpallewar et al., 2010), and all drugs were administered by intraperitoneal injection. All animals for gene expression analysis were sacrificed 2 h after the final injection. Nestin-GFP mice used for analysis of quiescent neural precursor populations were sacrificed 24 h following the last treatment.

2.2.2. Electroconvulsive seizure

To address the effects of electroconvulsive seizure (ECS) therapy on hippocampal gene expression, rats received ECS once daily *via* bilateral spring loaded ear clip electrodes (ECT unit, UGO Basile, Comerio, Italy) (Frequency: 100 pulses/s; pulse width: 0.9 ms; pulse duration: 0.5 s; current: 70 mA) or sham treatment (application of ear clip electrodes without electrical stimulation) for 7 consecutive days (n = 8–10 per group). All animals showed tonic clonic seizures in response to ECS administration. For gene expression analysis, rats were sacrificed 2 h after the final seizure administration. To examine the effects of ECS treatment on quiescent neural precursors (QNPs), nestin-GFP mice (n = 3–4 per group) received either a single ECS or sham treatment and were sacrificed 24 h later.

2.3. Microarray

Microarray analysis was performed to examine global transcriptional changes in the hippocampi of Y + I treated animals as compared to vehicle-treated controls (n = 3 per group). Hippocampi were dissected, frozen in liquid nitrogen, and stored at −80 °C prior to use. Hippocampal RNA was extracted using an RNeasy Minikit (Qiagen, The Netherlands) with RNA quality control assessment performed using the optical density ratio of 260/280 nm (NanoDrop spectrophotometer, NanoDrop Technologies, GE Healthcare, UK) and RNA integrity analysis performed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). RNA was labeled using the Agilent Quick Amp labeling kit (Agilent Technologies). The labeled RNA sample (800 ng) was fragmented and hybridized to a custom *Rattus norvegicus* array 8 × 60k (Agilent microarray design identifier: 028279). The hybridized slides were washed as per manufacturer's instructions and scanned using the Agilent microarray scanner G, model G2505C, at 3 μm resolution. Data extraction was performed using the Agilent Technologies Feature Extraction software (version 10.5.1.1). Data were then analyzed using GeneSpring GX, version 7.3.1, software from Agilent Technologies. Normalization of the data was done in GeneSpring GX using the one color per chip and per gene, data were as follows: (1) transformation: set measurements <0.01 to 0.01; (2) per chip: normalize to 50th percentile; (3) per gene: normalize to specific samples (control). Differentially regulated genes were filtered with a cutoff of >1.5 for upregulation and <0.66 for downregulation. Statistical analysis was done using a *t* test with a significance level of 0.05 and corrected for multiple comparisons using the Benjamini and Hochberg method (Benjamini and Hochberg, 1995). Hierarchical clustering was done based on fold change values for each gene using the GeneSpring software. Array data from Y + I treated animals as compared to vehicle treated controls have been deposited in the NCBI's Gene Expression Omnibus under the GEO series accession number GSE61301. Functional analysis of genes regulated by the Y + I treatment *versus* control was done using DAVID (Database for Annotation, Visualization, and Integrated Discovery; http://david.abcc.ncifcrf.gov/) functional annotation tool (Dennis et al., 2003; Huang et al., 2009).

2.4. Quantitative PCR

qPCR was performed to (1) validate candidate genes from our microarray analysis in an independent cohort of Y + I treated animals, and (2) to compare gene expression profiles following Y + I treatment with those evoked by the fast acting antidepressant, ECS and the slow-onset pharmacological antidepressant, imipramine (21 days I). Hippocampal tissue was dissected and RNA extraction was performed followed by quality control analysis as described in Section 2.3. RNA was then subjected to reverse transcription and subsequently qPCR analysis (Applied Biosystems, Thermo Fisher Scientific Corporation, USA). Quantification was performed using the ddCT method as described previously