Neuropharmacology 107 (2016) 329–338

Contents lists available at ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Rapid stress-induced transcriptomic changes in the brain depend on beta-adrenergic signaling

Martin Roszkowski [a], Francesca Manuella [a], Lukas von Ziegler [a], Gonzalo Durán-Pacheco [b], Jean-Luc Moreau [c], Isabelle M. Mansuy [a], Johannes Bohacek [a, *]

[a] Laboratory of Neuroepigenetics, Brain Research Institute, Neuroscience Center Zürich, University of Zurich/ETH Zurich, Winterthurerstrasse 190, 8057, Zurich, Switzerland
[b] Pharma Research and Early Development, Pharmaceutical Sciences, Translational Technologies and Bioinformatics, F. Hoffmann-La Roche Ltd, Basel, Switzerland
[c] Pharma Research and Early Development, Neuroscience, Ophthalmology and Rare Diseases, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland

ARTICLE INFO

Article history:
Received 9 February 2016
Received in revised form
21 March 2016
Accepted 25 March 2016
Available online 26 March 2016

Keywords:
Stress
Transcriptome
Norepinephrine
Noradrenaline
Hippocampus
Gene expression
Corticosterone
Corticotropin releasing hormone

ABSTRACT

Acute exposure to stressful experiences can rapidly increase anxiety and cause neuropsychiatric disorders. The effects of stress result in part from the release of neurotransmitters and hormones, which regulate gene expression in different brain regions. The fast neuroendocrine response to stress is largely mediated by norepinephrine (NE) and corticotropin releasing hormone (CRH), followed by a slower and more sustained release of corticosterone. While corticosterone is an important regulator of gene expression, it is not clear which stress-signals contribute to the rapid regulation of gene expression observed immediately after stress exposure. Here, we demonstrate in mice that 45 min after an acute swim stress challenge, large changes in gene expression occur across the transcriptome in the hippocampus, a region sensitive to the effects of stress. We identify multiple candidate genes that are rapidly and transiently altered in both males and females. Using a pharmacological approach, we show that most of these rapidly induced genes are regulated by NE through β-adrenergic receptor signaling. We find that CRH and corticosterone can also contribute to rapid changes in gene expression, although these effects appear to be restricted to fewer genes. These results newly reveal a widespread impact of NE on the transcriptome and identify novel genes associated with stress and adrenergic signaling.

1. Introduction

In humans, stressful life events can precipitate neuropsychiatric diseases such as anxiety disorders, posttraumatic stress disorder (PTSD) and depression (Breslau, 2009; Calhoon and Tye, 2015; Lupien et al., 2009). Also in most other mammals, acutely stressful experiences lead to rapid behavioral and cognitive changes. The molecular mechanisms underlying the rapid effects of stress are still unclear, but are known to depend on tissue-specific changes in gene expression in the brain (McEwen et al., 2015). Stress pathways and many stress-induced signals including hormones, peptides and neurotransmitters - here called stress mediators - are well characterized and highly conserved between humans and rodents (Joëls and Baram, 2009; Ulrich-Lai and Herman, 2009). Presumably, the complex transcriptomic changes that unfold over time following stress exposure are regulated by these stress mediators, likely with specific yet overlapping temporal profiles (Hermans et al., 2014; Joëls and Baram, 2009). The stress hormone corticosterone is the best characterized regulator of stress-induced gene expression (Datson et al., 2011; Morsink et al., 2006). However, corticosterone surges in the brain more than 20 min after stress exposure (Droste et al, 2008), thus contributing only minimally to the regulation of early stress-induced changes in gene expression (Bohacek et al., 2015b; Gray et al., 2014). In contrast to the slow corticosterone rise, there is a rapid central response to stress largely mediated by norepinephrine (NE) release from the locus coeruleus and by local release of corticotropin-releasing hormone (CRH) (Hermans et al., 2014; Maras and Baram, 2012). However, it remains unclear whether NE and CRH mediate the transcriptional changes observed shortly after stress. While the stress axis comprises many brain

* Corresponding author.
E-mail address: bohacek@hifo.uzh.ch (J. Bohacek).

http://dx.doi.org/10.1016/j.neuropharm.2016.03.046
0028-3908/

regions, stress-induced gene expression changes have been best characterized in the hippocampus (Gray et al., 2014; Irwin, 2001; Stankiewicz et al., 2015; Weaver et al., 2006), which is involved in the effects of stress on cognitive function, anxiety and affective disorders (Gobinath et al, 2015; Lupien et al., 2009).

Although severe stress is a risk factor for affective disorders in both sexes, the prevalence of these disorders is estimated to be 1.5–2-fold higher in women than in men (Tolin and Foa, 2006). Additionally, the transcriptional response to acute stress is often more pronounced in females than in males (Aloisi et al., 1997; Bohacek et al., 2015b). Nonetheless, most studies of stress-induced illnesses have focused exclusively on males, and females need to be included in basic science research to fully understand the mechanisms of stress and disease (Woodruff, 2014).

Here we analyze the transcriptomic response to an acute stress challenge in the hippocampus, and use a pharmacological approach to test which neurotransmitters and hormones are involved in this response. The analyses focus on females, but males were also tested when necessary to corroborate our conclusions. We find strong and widespread effects of acute swim stress on the transcriptome in the female hippocampus, with similar changes in males. We demonstrate that NE plays a predominant role in mediating gene expression after acute swim stress via β-adrenergic signaling. Corticosterone and CRH, however, have limited effects on the rapid changes in gene expression examined here. These results point to a profound, previously uncharacterized role for NE in the immediate effects of stress on the transcriptome.

2. Methods

2.1. Animals

C57Bl/6 J female and male mice (2.5 months) were obtained from Janvier (France) and maintained in a temperature- and humidity-controlled facility on a 12 h reversed light-dark cycle (lights on at 8:30 am) with food and water *ad libitum*. They were bred in-house to generate animals for the current experiments. After weaning, mice were housed in groups of 4–5 per cage and used for experiments when 2.5–6 months old. For each experiment, siblings of the same age were used in all experimental groups, to rule out confounding effects of age. All procedures were carried out in accordance to Swiss cantonal regulations for animal experimentation and were approved under licenses 55/2012 and 175/2013.

2.2. Stress paradigms

During all procedures, mice were monitored closely by a trained experimenter. For the initial microarray experiment, mice were single-housed immediately before stress/handling. For subsequent experiments, mice were single-housed 24 hrs before stress/handling, because this reduces stress hormone levels and avoids confounds of disturbing cage-mates on test day (Bohacek et al., 2015b). Immediately after stress/handling, mice were returned to their assigned single-housing homecage. Unless otherwise stated, mice were killed by cervical dislocation and decapitation 45 min after initiation of stress/handling.

2.2.1. Cold swim stress

Cold swim stress was conducted as described before (Bohacek et al., 2015b). Mice were placed in a plastic cylinder (18 cm high, 13 cm diameter) filled with 18 ± 1 °C water up to 12 cm height for 6 min. For control handling, mice were picked up by the base of the tail for about 4sec, to mimic the handling involved in the stress procedure.

2.2.2. Restraint stress

Restraint stress was conducted as described before (Bohacek et al., 2015b). Each mouse was placed in a 50 ml Falcon tube with a large air hole cut out for its nose for 6 or 30 min, as described in the results section. For cold restraint, the tube was tilted so that the nose-hole faced upwards, while the bottom of the tube was submerged in 18 ± 1 °C water for 6 min.

2.2.3. Footshock stress

For footshock stress, mice were placed in a fear conditioning apparatus (TSE Systems, Germany). After 3 min, they received a single footshock (1.5 mA, 2sec), and were removed from the chamber 30 s after termination of the footshock.

2.3. Drugs

All drugs were mixed fresh the morning of the experiment. Mifepristone (Sigma, M8046) and antalarmin hydrochloride (Sigma, #A8727) were dissolved in DMSO and diluted into 0.9% saline (20% DMSO). Propranolol (Sigma, #P0884), betaxolol (Tocris, #0906), and ICI118,551 (Tocris, #0821) were each dissolved in 0.9% saline. Vehicle injections were either 0.9% saline, or saline containing 20% DMSO. All injections were administered i.p., injection volume was 10 μl per gram body mass. Doses were selected based on previous reports as indicated in the results section.

2.4. Molecular analyses

2.4.1. Tissue collection and processing

Tissue processing was conducted as described before (Bohacek et al., 2015b). Immediately after sacrifice, the brain was removed and the whole hippocampus, prefrontal cortex, olfactory bulbs and cerebellum were rapidly dissected on ice and stored at −80 °C until processing. RNA was extracted using Trizol (Invitrogen) according to manufacturer's recommendations and on-column DNase treated using the DNase-free RNA kit (Zymo Research). cDNA libraries were generated using Superscript III (Invitrogen), following manufacturer's recommendations, and stored at −20 °C until further analyses.

2.4.2. Gene expression microarrays

Adult mice were sacrificed by cervical dislocation 45 min after initiation of cold swim stress (6 min, 18 °C water). The brain was removed and the hippocampus rapidly dissected on ice and stored at −80 °C until further processing. RNA was extracted with RNeasy spin columns (Qiagen) and amplified using the Ovation RNA amplification kit V2 (Nugen Technologies). RNA was labeled, hybridized and analyzed using NimbleGen Mouse Gene Expression 12 × 135 K Arrays according to manufacturer's recommendations. Each RNA sample (n = 8/group) was hybridized to two independent NimbleGen slides for technical replication of each measurement. Principal Component Analysis (PCA) demonstrated high reproducibility between technical replicates and subsequent analyses were conducted as described previously (Bohacek et al., 2015a). Briefly, raw data were processed with RMA (Robust Multichip Average) according to NimbleGen's recommendation. For analysis of differential expression, the expression matrix was log2 transformed and imported into Partek Genomics Studio (Partek Inc, Missouri, USA) and Limma (Smyth, 2005). A linear model was run to identify probes for genes differentially expressed between groups and multiple testing corrected p-values (FDR method) were calculated. Heatmaps were generated using free online software (CIMminer, http://discover.nci.nih.gov/cimminer/home.do). For KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis, all genes with FDR-corrected p-values < 0.05 were selected