European Journal of Cell Biology 89 (2010) 712–722

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.de/ejcb

Dexamethasone enhances the norepinephrine-induced ERK/MAPK intracellular pathway possibly via dysregulation of the α_2-adrenergic receptor: Implications for antidepressant drug mechanism of action

Shiri P. Yaniv, Anat Lucki, Ehud Klein, Dorit Ben-Shachar*

Laboratory of Psychobiology, Department of Psychiatry, Rambam Medical Center and B. Rappaport Faculty of Medicine, Technion, Haifa, Israel

ARTICLE INFO

Article history:
Received 10 December 2009
Received in revised form 16 May 2010
Accepted 17 May 2010

Keywords:
Norepinephrine
glucocorticoids
ERK/MAPK
plasticity
CREB
β-arrestin

ABSTRACT

Norepinephrine (NE) and glucocorticoids (GCs) have been shown to oppositely affect various aspects of neuronal plasticity. These findings provided the basis for the plasticity hypothesis of major depression, which suggests that the disease-related impairment in neuronal plasticity is associated with long-term increase in GCs and may be reconstituted by antidepressants and monoamines. To investigate the interaction between GCs and NE, the plasticity-relevant ERK/MAPK pathway was studied in SH-SY5Y neuroblastoma cells treated with dexamethasone (DEX), a synthetic GC, NE, or both. NE treatment activated ERK and c-Jun and increased AP-1 transcriptional activity. Although DEX had no effect, co-treatment caused a prolonged and robust activation of the ERK/AP-1 pathway beyond NE-induced activation. Co-treatment also induced hyperactivation of CREB as compared to NE activation while DEX decreased pCREB. Independent alterations of ERK and CREB suggest an upstream point of interaction. Yohimbine, an α_2-adrenergic receptor (AR) antagonist, prevented the hyperactivation of the ERK/AP-1 pathway and CREB induced by co-treatment. Immunofluorescence showed that after 2 h of NE treatment, β-arrestin was co-localized with the α_2-AR at the plasma membrane while following co-treatment β-arrestin was diffused within the cell, suggesting that DEX delays AR downregulation by altering β-arrestin translocation. These results show a novel complex interaction by which GCs augment NE-induced intracellular signaling that may be relevant to antidepressant mode of action.

Introduction

Norepinephrine (NE) has been shown to induce several aspects of neuronal plasticity including neurite sprouting and upregulation of brain derived neurotrophic factor (BDNF) expression (Juric et al., 2006) as well as additional plasticity related genes such as CREB, the adhesion molecule CAM-L1, and growth-associated protein 43 (Gap-43) in cell culture (Laifenfeld et al., 2002). Oppositely, glucocorticoids (GCs) have the ability to reduce neuronal plasticity (review Duman et al., 1999) by triggering dendritic atrophy and neurotoxicity (Yu et al., 2004), as well as by affecting the expression of several plasticity related genes such as CREB and BDNF (Laifenfeld et al., 2005b; Yu et al., 2004). These alterations in neuronal plasticity form the basis of the plasticity hypothesis of major depression (MD) which states that the decrease of neuronal plasticity is due to high GC levels and can be reversed by antidepressants as well as by monoamines (Duman et al., 1999). Antidepressants can also increase neuronal plasticity exhibited as morphological alterations in rat models (Malberg et al., 2000) and gene expression in both humans and in animal models (Dowlatshahi et al., 1998; Laifenfeld et al., 2005a). The therapeutic mechanism of action of most antidepressants is attributed to their ability to increase the level of monoamines, mainly serotonin (5-HT) and NE, in the synapse, suggesting monoamines can modulate neuronal plasticity.

One possible mechanism by which NE and GCs exert their opposite effects on neuronal plasticity is via the mitogen-activated protein kinase (MAPK) intracellular pathway. NE has been shown to activate the MAPK pathways and activator protein-1 (AP-1), the downstream transcription factor of ERK (Alblas et al., 1993; Zhong and Minneman, 1999), while GCs may inhibit AP-1 activity by disrupting transcriptional activity (Jonat et al., 1990) and by downregulating c-Fos expression (Tsitoura and Rothman, 2004). In addition, GCs can upregulate MAPK phosphatase (MKP)-1 which

Abbreviations: 5-HT, serotonin; AP-1, activator protein 1; AR, adrenergic receptor; BDNF, brain derived neurotrophic factor; DEX, dexamethasone; FCS, fetal calf serum; GC, glucocorticoid; GPCR, G-protein-coupled receptor; GR, glucocorticoid receptor; GRK, G-protein receptor kinase; MAPK, mitogen-activated protein kinase; MD, major depression; MKP, MAPK phosphatase; MR, mineralocorticoid receptor; NE, norepinephrine; SSRI, specific serotonin re-uptake inhibitor.

* Corresponding author at: Laboratory of Psychobiology, Department of Psychiatry, Rambam Medical Center and B. Rappaport Faculty of Medicine, Technion ITT, POB 9649, Haifa 31096, Israel. Tel.: +972 4 8295224; fax: +972 4 8295220.
E-mail address: shachar@tx.technion.ac.il (D. Ben-Shachar).

0171-9335/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ejcb.2010.05.002

inactivates the MAPK pathways (Kassel et al., 2001), thereby decreasing signaling pathway efficacy and AP-1 activation. Supporting the relevance of this pathway to NE and GCs induced alteration in plasticity is evidence linking the ERK pathway to various plasticity processes including neurogenesis, modulation of dendritic and axonal growth, and the regulation of BDNF levels (Chen and Manji, 2006). Alterations in the ERK/MAPK pathway such as decreased ERK expression and activation have been seen in postmortem samples of MD patients (Dwivedi et al., 2001, 2009), a finding paralleled in rat models (Feng et al., 2003). In addition, treatment of rats with an ERK inhibitor produces MD-like behaviors and blocks antidepressant action (Duman et al., 2007) while antidepressants increase the expression of AP-1 (Hope et al., 1994; Morinobu et al., 1997). Finally, CREB, an additional important transcription factor implicated in MD (Dwivedi et al., 2003) and in antidepressant treatment (Tardito et al., 2009) that plays a considerable role in several aspects of plasticity (Tardito et al., 2006) can also be regulated by the ERK pathway (Roberson et al., 1999).

These findings provide a basis for possible interactions between NE and GCs on the MAPK, an interaction that has not yet been studied. We have previously demonstrated opposite effects of NE and GCs on several plasticity related parameters, including morphology and expression of plasticity related genes which can be regulated by the MAPK pathways, in cell culture (Yaniv et al., 2008). Therefore, in the present study we examined the effects of treatment with NE and dexamethasone (DEX), a synthetic GC receptor agonist, on the MAPK pathways in SH-SY5Y human neuroblastoma cells. Discovering the molecular interaction between GCs and NE, both of which play an important role in major depression and its treatment, will aid in understanding the underlying mechanism by which antidepressants restore plasticity following stress.

Materials and methods

Human neuroblastoma cells

SH-SY5Y were grown at 37 °C, with 5% CO_2 in Dulbecco's Modified Eagle's Medium (DMEM) with 4.5 g/l D-glucose supplemented with 2 mM L-glutamine, 100 U/ml streptomycin/penicillin and 10% FCS (Biological Industries, Kibbutz Bet-Haemek, Israel). Culture medium was changed every 72 h.

HPLC analysis

Cells were treated with 10^{-6} M desipramine (DMI), 10^{-5} M DEX or both for 1 h. The medium was collected and $HClO_4$ was added to a final concentration of 0.1N. Cells were washed twice with cold PBS and suspended in 0.1N $HClO_4$. After centrifugation 12,000 × *g* for 15 min the supernatant was collected and stored at −80 °C until used.

Dialysate samples were analyzed for NE, 3,4-dihydroxyphenylethylene glycol (DHPG) and 3-methoxy-4 hydroxyphenylethylene glycol (MHPG) by HPLC with electrochemical detection. Separation of NE and its metabolites was achieved using an Inertsil ODS-2 column (GL Sciences, Japan) with a mobile phase composed of 100 mM sodium dihydrogen phosphate, 1.46 mM octanesulfonic acid, 2.3% (v/v) methanol, 4.15% (v/v) acetonitrile, 248 μM sodium EDTA in HPLC grade deionized water, pH 2.75. Detection of compounds was enabled by an ESA coulochem II (model 5200, ESA, USA) operated in redox mode. Column eluates were initially oxidized at a potential of +300 mV using an ESA guard cell placed before the detector, reduced to +100 mV at detector 1 of the analytical cell (ESA model 5010), and measured at −400 mV at detector 2. The limit of detection for NE, DHPG and MHPG was 0.01 pmol.

Drug treatment

Cells (0.5×10^5 per 2.5 mm plates) were incubated with growth medium for 24 h, after which the medium was replaced with serum-free medium. 18 h later cells were treated with a solution of 10^{-5} M NE, 10^{-5} M DEX, 10^{-6} M DMI, 10^{-5} M NE + 10^{-5} M DEX, or 10^{-6} M DMI + 10^{-5} M DEX in PBS (Sigma–Aldrich, St. Louis, MO, USA) for 10 min up to 48 h. Control cells were incubated in an equivalent volume of PBS for the same length of time. 50 μM of MEK inhibitors PD98059 and U0126 dissolved in DMSO (Sigma–Aldrich, St. Louis, MO, USA), were administered to cells 30 min before drug treatment. Control cells were incubated in an equivalent volume of DMSO for the same length of time.

Protein extraction and Western Blotting

Total protein was extracted from cells using RIPA buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 2 mM EDTA, 1% NP-40 and protease and phosphatase inhibitors (Complete™ protease inhibitor cocktail tablets, Roche Diagnostics, Mannheim, Germany; phosphatase inhibitor cocktails I and II, Sigma–Aldrich, St. Louis, MO, USA). Protein concentration in the supernatant of cell lysate was measured using a modified Bradford protein assay (Zor and Selinger, 1996). Protein (80 μg) was prepared with 2× sample buffer (Sigma–Aldrich, St. Louis, MO, USA), run on a 10% SDS-PAGE and transferred to a nitrocellulose membrane. Quality of transfer was assayed by Ponceau staining (Sigma–Aldrich, St. Louis, MO, USA). Membranes were blocked in 5% non-fat milk (Bio-Rad, Hercules, CA, USA) for 2 h at room temperature and then incubated overnight at 4 °C with the appropriate primary antibody. Following washes, the membrane was incubated with the corresponding secondary antibody for 1 h at room temperature. Membranes were developed with ECL reagents (Amersham Biosciences) and exposed to XLS Kodak film. Films were analyzed by densitometer (Vilber-Lourmat, France), using β-actin levels for normalization. Primary antibodies used were: pERK1/2 (E-4, Tyr204, 1:100), ERK1 (K-23, 1:800), p-c-Jun (Ser63, 1:100), c-Jun (N, 1:400), p-p38 (Tyr182, 1:500), p38 (A-12, 1:400), c-Fos (4, 1:200), CREB-1 (C-21, 1:500), JNK1 (C-17, 1:300), MKP-1 (C-19, 1:300), p-ATF2 (Thr69/71, 1:100) and ATF-2 (N-96, 1:200) purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA); pCREB (Ser133, 1:500) and p-SAPK/JNK (Thr183/Tyr185, 1:1000) were purchased from Cell Signaling Technologies (Boston, MA, USA) and β-actin (1:10,000) purchased from Sigma–Aldrich (St. Louis, MO, USA).

Transfections and reporter assay

Transient transfections of SH-SY5Y cells with 1 μg pAP1-Luc plasmid (Clontech, Mountain View, CA, USA) were carried out using FuGENE 6 (1:6, w/v) (Roche Diagnostic Hoffmann-La Roche Ltd., Basel, Switzerland). Luciferase assay was performed 1 h, 6 h, 24 h and 48 h after treatment with 10^{-5} M NE, 10^{-5} M DEX, 10^{-5} M NE + 10^{-5} M DEX, or 10 nM Phorbol 12-Myrsitate 13-Acetate (PMA) (Sigma–Aldrich St. Louis, MO, USA), using luciferase assay kit (Promega, Madison, WI, USA). Luciferase activity was measured by microplate luminometer (Anthos Lucy 1, Anthos Labtec Instruments, Salsburg, Austria). Cells transfected with empty vector pTal-Luc (Clontech, Mountain View, CA, USA) served as a control.

cAMP levels

Intracellular cAMP levels were measured after treatment for 10 min or 30 min with 10^{-5} M NE, 10^{-5} M DEX or 10^{-5} M NE + 10^{-5} M DEX using the acetylated version of the Direct cAMP Enzyme Immunoassay Kit (Sigma–Aldrich St. Louis, MO, USA).