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Research paper

Interdependent adrenergic receptor regulation of Arc and Zif268 mRNA in cerebral cortex

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HIGHLIGHTS

- α_2-AR uniquely exert an inhibitory regulation of both Arc and Zif268 compared to α_1 and β-AR.
- The α_2-AR antagonist, RX821002, increases Arc and Zif268 interdependent with α_1 and β-AR.
- A lack of adult IEG response to combined α_1 and β-AR blockade contrasts with decreases to *n*-(2-chloroethyl)-*n*-ethyl-2-bromobenzylamine hydrochloride (DSP4).

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ABSTRACT

Norepinephrine is a neurotransmitter that signals by stimulating the α_1, α_2 and β adrenergic receptor (AR). We determined the role of these receptors in regulating the immediate early genes, Activity Regulated Cytoskeleton Associated Protein (Arc) and Zif268 in the rat cerebral cortex. RX821002, an α_2-AR antagonist, produced Arc and Zif268 elevations across cortical layers. Next we examined the effects of delivering RX821002 with an α_1-AR antagonist, prazosin, and a β-AR antagonist, propranolol. RX821002 given with a prazosin and propranolol cocktail, or with each of these antagonists individually, decreased Arc and Zif268 to saline-treated control levels in most cortical layers. Arc and Zif268 levels were also similar to saline-treated control levels when rats were given a prazosin and propranolol cocktail alone, or when each of these antagonists were delivered individually. Taken together, these data reveal that α_2-AR uniquely exert a tonic inibitory regulation of both Arc and Zif268 compared to α_1 and β-AR. However, the ability of RX821002 to increase Arc and Zif268 is interdependent with α_1 and β-AR signaling.

1. Introduction

Norepinephrine is a neurotransmitter that works by stimulating the α_1, α_2 and β adrenergic receptor (AR) [1]. Signaling through ARs serves critical modulatory roles for cortical plasticity [2–4]. For example, ocular dominance plasticity is facilitated by infusing norepinephrine into the developing occipital cortex [2]. Other investigations have shown that increasing norepinephrine levels assists in functional recovery from brain injury [3]. Conversely, destroying noradrenergic innervation impairs the ability of an animal to recover from cortical injury, relative to animals with noradrenergic systems intact [4].

Norepinephrine's influential roles in cortex may lie, in part, in its regulation of immediate early gene (IEG) expression [5–9]. Activity Regulated Cytoskeleton Associated Protein (Arc), for instance, is classified as an IEG due to its rapid activation by robust synaptic stimulation through long term potentiation (LTP) and memory-related behavioral paradigms [10]. Arc is unique since neuronal activity results its rapid enrichment within dendrites of the cortex and hippocampus [11,12]. In the cerebral cortex Arc plays an important role in experience-dependent homeostatic plasticity of excitatory synaptic transmission [13], and in consolidating the activity of neural networks involved in motor learning [14].

Another critical IEG for brain function is Zif268, which is also critical for LTP and long-term memory formation [15]. Zif268 may also be important for endowing the brain with resiliency and

Abbreviations: Arc, activity regulated cytoskeleton associated protein; AR, adrenergic receptor; IEG, immediate early gene; LTP, long term potentiation; PBS, phosphate-buffered saline; RX821002, 2-[2-(2-methoxy-1,4-benzodioxanyl)]imidazoline hydrochloride; DSP4, *N*-(2-chloroethyl)-*n*-ethyl-2-bromobenzylamine hydrochloride.

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repair capacity. For example, Zif268 is decreased in the cortex of depressed humans and in mouse models of depression [16]. Noradrenergic lesions deplete Zif268 [8,9], and also cause the cortex to be vulnerable to neurotoxic insult [17]. Alternatively, optogenetic stimulation increases cortical Zif268 levels in mice in association with an antidepressant effect [16]. The overexpression of Zif268 also accompanies long-term functional recovery after cortical injury [18].

Given the potential importance of Arc and Zif268 to norepinephrine's regulation of cortical plasticity, we further characterized the noradrenergic regulation of these critical IEGs. For example, RX821002 is a α_2-AR receptor antagonist that induces brain IEG expression [5,19], demonstrating that α_2-AR exert a tonic inhibitory regulation of IEG levels. In contrast to α_2-AR, the role that α_1 and β-AR play in regulating basal Arc expression, however, has not been examined. Moreover, although ARs frequently work in coordinated manner to regulate plasticity [20–22], how α_1, α_2 and β-AR integrate their signaling to modulate Arc and Zif268 has not been well studied.

In these experiments cortical Arc and Zif268 expression were therefore measured after delivering individual as well as combined AR antagonist treatments. Our data reveal distinctive roles for α_2 versus α_1 and β-AR in regulating Arc and Zif268. These data further characterize a coordination of these receptors to modulate levels of these key IEGs.

2. Materials and methods

2.1. Animals and adrenergic receptor antagonist treatment

Sprague-Dawley rats (Sasco, Kingston, NY) were bred in our colony. Rats (250–300 g) received i.p. injections of vehicle alone or drugs at the following dosages: RX821002 (α_2-AR antagonist, 5 mg/kg), prazosin (α_1-AR antagonist, 15 mg/kg), propranolol (β-AR antagonist, 10 mg/kg) (n = 4–6 animals per group). RX821002 and propranolol were dissolved in sterile saline immediately prior to use while prazosin was dissolved in 75% glycerol. After injection, animals were allowed to sit quietly in their home cage for 1 h and then taken to a separate room where they were killed by decapitation under isoflourane anesthesia and brains were removed. Animals were handled for twice a day for 2 days prior to injection in order to minimize the effects of injection stress alone. All animal use procedures were in strict accordance with The National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Nebraska Medical Center Animal Care and Use Committee.

2.2. In situ *hybridization*

Sixteen micron tissue sections were cut in a cryostat and thaw-mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). Sections were fixed in ice cold 4% paraformaldehyde in phosphate buffered saline (PBS) pH 7.4, for 5 min, washed in PBS for 1 min and in 75% ethanol for 2 min, and stored in 100% ethanol at 4 °C. Slides were allowed to dry at room temperature prior to *in situ* hybridization. Oligonucleotide probe sequences were as follows; Arc: 5′-CTT-GGT-TGC-CCA-TCC-TCA-CCT-GGC-ACC-CAA-GAC-TGG-TAT-TGC-TGA-3′ (complementary to bases 789–833, NM_019361.1) and zif268: 5′-CCG-TTG-CTC-AGC-AGC-ATC-ATC-TCC-TCC-AGT-TTG-GGG-TAG-TTG-TCC-3′ (complementary to bases 355–399, NM_012551.1). A Blast search of Genbank found that these sequences do not have significant homology with any other sequences. Probes were 3′ end labeled with [^{35}S]-dATP (1200 Ci/mmol, PerkinElmer, Boston, MA) using terminal deoxyribonucleotidyl transferase (3′ End Labelling System, PerkinElmer). Labeling reactions were purified of unincorporated radionucleotide with Biospin-6 chromatography columns (BioRad, Hercules, CA) by centrifuging at 2500 rpm for 5 min. Hybridization buffer (150 μl of 50% formamide, 4XSSC, 8% dextran sulfate, 1XDenhardt's, 500 μg/mL salmon sperm DNA, 270 μg/mL yeast tRNA, 0.1 mM DTT) containing 1×10^6 cpm of labeled probe was applied to each slide. Non-specific labeling was determined by inclusion of 10X unlabeled probe. Slides were coverslipped, sealed with D.P.X. (Aldrich Chemical Co., Milwaukee, WI) and placed overnight in a 1XSSC humidified sealed Tupperware container at 42 °C. The next day coverslips were removed in 55 °C 1XSSC and slides were washed 4 × 15 min in 1XSSC at 55 °C. After washing, slides were briefly dipped in room temperature water to remove salts and in 70% ethanol to facilitate drying. Slides were apposed to Biomax film (Kodak, Rochester, NY) for 2–3 weeks. Films were developed using standard techniques and analyzed using the MCID-M7 image analysis system (Interfocus Imaging, Ltd., Linton, England).

2.3. Image analysis

Autoradiographic densities were quantified using commercial tritium standards (American Radiochemicals, St. Louis, MO) that were previously calibrated to ^{35}S [23]. Cortical expression was measured at a coronal level 0.7 mm anterior to the bregma.

2.4. Statistics

Studies examining the effects of adrenergic antagonists on IEGs across cortical layers were analyzed by two-way ANOVA with the Bonferonni posthoc test. For graphical presentation these data are presented as percentage increase over the average basal expression level.

3. Results

3.1. RX821002 produces increases in Arc and Zif268 that are blocked by a prazosin and propranolol cocktail

In these experiments we first studied the response of Arc and Zif268 to RX821002 (α_2-AR antagonist) delivered alone, or with prazosin (α_1-AR antagonist) and propranolol (β-AR antagonist) as a cocktail. We also tested the response of Arc and Zif268 to a prazosin and propranolol cocktail alone.

We found that Arc was rich in layers 4 and 6 of cortex, and showed many changes to adrenergic receptor antagonist treatment. Statistical analysis showed an effect for both cortical layer ($F_{(3,152)}$ = 13.74, p < 0.001) and drug treatment ($F_{(3,152)}$ = 81.43, p < 0.0001). Injecting RX821002 caused a ~50% increase in Arc expression across cortical layers. In contrast, to increases in Arc

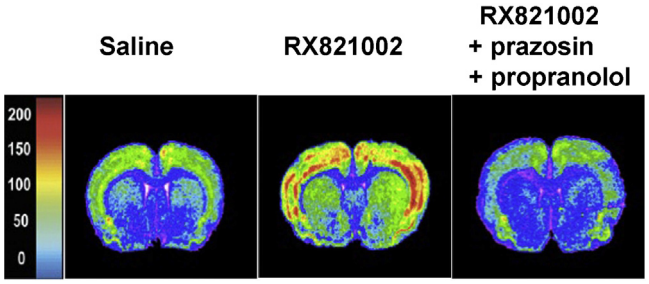

Fig. 1. *In situ* hybridization autoradiographs for Arc. These images correspond to 0.7 mm anterior to bregma in adult animals. The greatest enrichment for Arc mRNA is seen in layers 4 and 6. RX821002 causes increases in Arc mRNA that are blocked by co-administration of prazosin and propranolol. The scale bar indicates the density of mRNA calibrated for nCi/mg tissue.