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ANTAGONISTIC INTERACTIONS BETWEEN DEXAMETHASONE AND FLUOXETINE MODULATE MORPHODYNAMICS AND EXPRESSION OF CYTOKINES IN ASTROCYTES

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Abstract—The ''plasticity hypothesis'' proposes that major depression is caused by morphological and biochemical modifications in neurons and astrocytes and those beneficial pharmacological effects of selective-serotonin-reuptake-inhibitors (SSRI) are at least partially associated with modifications of cellular communications between these cells. In this study we examined effects of the antidepressant fluoxetine on cultured astrocytes that were, in some cases, pretreated with dexamethasone, a cortisol analog known to trigger depressive disorder. Primary rat astrocytes were purified and treated with dexamethasone and the SSRI fluoxetine in physiological concentrations so that both drugs did not affect cell viability. Expression of interleukin-2 (IL-2) and glia-derived-neurotrophic-factor (GDNF) were analyzed and monitored and cell viability, apoptosis, cluster formation, particle-removing capacity and cell mobility were also monitored. Pre-studies without any drugs on mixed neuron-astrocyte co-cultures suggested that astrocytes interacted with neurons and other brain cells *in vitro* by actively assembling them into clusters. Treatment of purified astrocytes with dexamethasone significantly decreased their mobility compared to controls but had no effect on cluster formation. Dexamethasone-treated cells removed fewer extracellular particles derived from dead cells and cell debris. Both effects were abolished by simultaneous application of fluoxetine. Intracellular IL-2 increased, while GDNF amount expression was diminished following dexamethasone treatment. Simultaneous administration of fluoxetine reversed dexamethasone-triggered IL-2 elevation but had no effect on decreased GDNF concentration. These results suggest that mobility and growth factor equilibrium of astrocytes are affected by dexamethasone and by fluoxetine and that fluoxetine could reverse some changes induced by dexamethasone.
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Key words: antidepressant, astrocytes, dexamethasone, fluoxetine, depression.

INTRODUCTION

Effects of antidepressants on neurons have been analyzed in several studies and results from these studies have triggered the formulation of the so-called ''plasticity hypothesis of depression'' (Kempermann and Kronenberg, 2003). This evidence suggested that pathological modifications of neuronal and synaptic connections were the cause of depressive disorder in humans (Duman et al., 1997), rather than a deficit of certain neurotransmitters as proposed by the ''monoamine hypothesis'' (Schildkraut, 1965). A major argument for the plasticity hypothesis is that the antidepressant tianeptine enhances monoamine uptake but is even more effective treating depression than monoamine uptake inhibitors (Mennini et al., 1987; McEwen et al., 2010). Nevertheless, a unifying hypothesis for a general mechanism of antidepressant action is still not in sight yet. Recent findings have shifted focus to astrocytes which are potential targets for antidepressant drugs (Czeh et al., 2006), since it was found that their number and the expression pattern of astrocyte-derived regulatory proteins is significantly decreased in patients suffering from major depression (Rajkowska and Stockmeier, 2013). Animal models of depression also showed that stress-induced cellular, metabolic deficits in astrocytes were reduced by treatment with the glutamate-modulating drug riluzole, extending research to a new target for antidepressant treatment strategies (Banasr et al., 2010).

Motility of astrocytes and their interaction with neurons play an important role in the brain plasticity. Astrocytes express receptors for neurotransmitters, including serotonin receptors (type 5-HT2B), which are a major target for selective-serotonin-reuptake-inhibitors (SSRIs) (Peng and Huang, 2012).

Therapeutically relevant concentrations of fluoxetine activate the PI3K-AKT-GSK-3β pathway and ERK1/2 kinases, thereby increasing gene expression of c-fos and fosB (Li et al., 2008; Zhang et al., 2010). Astrocyte-derived neurotrophins are essential for neuronal plasticity, and their expression is increased by antidepressants (Allaman et al., 2011; Song and Wang, 2011). Astrocytes are also an important source of growth factors involved in neuronal plasticity and pathophysiology of major depres-

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Abbreviations: ANOVA, analysis of variance; BDNF, brain-derived neurotrophic factor; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GDNF, glia-derived-neurotrophic-factor; GFAP, glial fibrillary acidic protein; IL-2, interleukin-2; SD, Sprague–Dawley; SERTs, serotonin transporters; SSRI, selective-serotonin-reuptake-inhibitors.

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sion (Kajitani et al., 2012; Quesseveur et al., 2013b). There is evidence that glia cells show disease-specific pathology (Schroeter et al., 2008). Immunohistology studies conducted on postmortem brains revealed that expression of glial fibrillary acidic protein (GFAP) was significantly reduced in the prefrontal cortex in depressed patients (Si et al., 2004). A similar finding was revealed for brain-derived neurotrophic factor (BDNF) (Quesseveur et al., 2013a), which is the most thoroughly investigated neurotrophic factor associated with the pathology of depression (Duman et al., 1997; Garcia, 2002; Lang et al., 2006). Although basal levels of BDNF in astrocytes are very low, these cells express, bind, store and release BDNF and act as a buffer system for this growth factor (Rubio, 1997; Wu et al., 2004; Jean et al., 2008). Apart from BDNF, there are several other neurotrophic factors which are regulated by antidepressants (Henkel et al., 2008). One of these is interleukin-2 (IL-2), a cytokine that was found to be increased in patients with depression (Yoon et al., 2012). Therefore we were interested if this inflammatory factor was unregulated in astrocytes by dexamethasone since it was found that immune-modulatory cytokines could be co-factors to enhance or even trigger depression (Mueller and Schwarz, 2007).

Major depression seems to induce cell-specific changes that include reductions in densities of astrocytes and oligodendrocytes in the prefrontal cortex, hippocampus, amygdale, raphe nucleus and locus coeruleus (Hercher et al., 2009). It is known that chemical stress factors like dexamethasone (Pariante, 2009) and α-interferon (Kenis et al., 2010) can trigger major depression in humans. These drugs also induce functional defects in cellular pathways in neurons *in vitro*, including inflammation, oxygen radical damage and neurodegeneration (Maes et al., 2009).

In this study, we investigated effects of fluoxetine and dexamethasone on astrocyte mobility, dynamic morphology and expression of IL-2 and glia-derived-neurotrophic-factor (GDNF). Analysis of associated cellular pathways and functions could provide information on mechanisms driving the organization of glial cell networks *in vitro* and might subsequently explain large-scale morphological modifications apparent in the brain of patients suffering from major depression (Sheline et al., 2012).

EXPERIMENTAL PROCEDURES

Animals

One- to 3-day-old Sprague–Dawley (SD) rats of both sexes were used to generate primary cultures of astrocytes. Animal care and handling procedures complied with standards of the International Council of Laboratory Animals Sciences and was approved by the Kuwait University Research Administration Ethics Committee. SD rats were sacrificed by cervical dislocation and purified primary astrocytes cultures were generated as described earlier (Redzic et al., 2010; Abbott and Friedman, 2012)

Preparation of primary astrocyte-neuron co-cultures

Hippocampal cells from 2-day-old SD rats were isolated according to a standard protocol with minor modifications described previously (Henkel et al., 2010). Cortical cells, composed mainly of astrocytes and neurons, were allowed to rest for 24 h and then imaged by a Cell Observer® cell culture incubation microscope (Zeiss, Jena, Germany) over a period up to 3 days.

Experimental groups and drug treatments

Fig. 1A shows an overview of the complete experimental procedure. Thirteen-day-old primary cultures were divided into four experimental groups and cultured for 7 days in Dulbecco's modified eagles medium (DMEM) containing 5.5 mM glucose that was supplemented with FCS (10%) and streptomycin (50 μg/ml). Subsequent drugs were added to the groups using the following protocol: Control group (no drugs); DEX group (dexamethasone 0.5 μM, (Takahashi et al., 2012)); FLUO group (no drug for 4 days, then 1 μM fluoxetine for 3 days); DEX/FLUO group (0.5 μM dexamethasone for 7 days, 1 μM fluoxetine for the last 3 days of treatment).

Subcellular fractionation

At the end of each treatment, subcellular fractionation of the cultured astrocytes was performed, following a modified standard procedure (Michaelson and Whittaker, 1963). Briefly, cells were detached and homogenized in ice-cold buffer containing 1 μg/ml leupeptin, 100 μg/ml bacitracin, 2 μg/ml aprotinin an 1 μg/ml pepstatin, 10% glycerol, 1.5 mM EDTA, 10 mM Tris, pH 7.4. Three fractions were separated using a two-step centrifugation protocol: P1, which consisted of nuclei, large fragments of membranes and large cytoskeleton scaffolds; P2, which contained large organelles and intracellular membranes; and S2 that contained soluble proteins, mitochondria and micro-vesicles.

P1 and P2 fractions were re-suspended in 100 μl of PBS and all samples, were stored at −20 °C. Protein concentrations in the S2 fraction were determined by the Bradford assay (Bradford, 1976) and in the P1, P2 fractions by an amido-black assay that excludes interference of membrane lipids with protein estimation (Henkel and Bieger, 1994).

Morphodynamic analysis

Astrocyte mobility was quantified from image stacks obtained on a Cell Observer (Zeiss, Germany) incubation microscope, controlled with AxioVision Basic Software. Image time-lapse series were taken every 30 min for up to 48 h, and images were subsequently aligned using the ImageJ 1.45 (National Institute of Health (NIH), Bethesda, Maryland, USA) module "stack rag", which was set on "translation transformation" mode. The image stacks were further processed by "SynoQuant 3.26" (self-written image analysis software) to normalize variable contrast and illumination changes and smoothed by morphological filtering using build-in batch functions. The mobility of the cells was measured

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