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Short communication

Striatal but not frontal cortical up-regulation of the epidermal growth factor receptor in rats exposed to immune activation in utero and cannabinoid treatment in adolescence

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ABSTRACT

In utero maternal immune activation (MIA) and cannabinoid exposure during adolescence constitute environmental risk factors for schizophrenia. We investigated these risk factors alone and in combination ("two-hit") on epidermal growth factor receptor (EGFR) and neuregulin-1 receptor (ErbB4) levels in the rat brain. EGFR but not ErbB4 receptor protein levels were significantly increased in the nucleus accumbens and striatum of "two-hit" rats only, with no changes seen at the mRNA level. These findings support region specific EGF-system dysregulation as a plausible mechanism in this animal model of schizophrenia pathogenesis.

1. Introduction

Numerous studies have implicated EGF-system dysfunction in schizophrenia with regards to its ligands such as EGF (Futamura et al., 2002) neuregulin-1 (Stefansson et al., 2002; Williams et al., 2003) and betacellulin (Schwarz et al., 2012); their cognate receptors EGFR (Futamura et al., 2002) and ErbB4 (Hahn et al., 2006; Pitcher et al., 2011; Stefansson et al., 2002); and associated signalling molecules such as PI3K p110δ isoform (Law et al., 2012). A number of aetiological risk factors for schizophrenia have also been identified including MIA (as result of maternal infection during pregnancy) and peri-adolescent cannabis use. However, it is not known how these factors may interact to mediate their increased risk for the disorder. We postulated that these two environmental risk factors may perturb the EGF-system to exert their effects. To test this we used a "two-hit" animal model in which rats were exposed to the viral mimetic, polyriboinosinic: polyribocytidylic acid (polyIC) in utero and the synthetic cannabinoid CB1 receptor (CB1R) agonist (HU210) in adolescence. Although selective CBIR agonist such as HU210 does not reflect the range of cannabinoids present in marijuana, it is an active component of some available synthetic cannabinoid preparations. Additionally it allows more focussed examination of the role of CB1R in influencing the EGF-system in the adult brain. We then measured a number of EGF-system components including EGFR, ErbB4 and PI3K p110δ in adult brains of these animals to investigate if these environmental factors perturb EGF-system signalling as seen in schizophrenia. The cortex was examined given its link with cognitive, negative and deficit syndrome symptoms in schizophrenia and connection with ErbB signalling through innervation by mesocortical dopamine tracts and glutamatergic and gamma-amino-butyric acid (GABA)-ergic neurons (Stefansson et al., 2002), whilst changes in ErbB signalling have been shown in the striatum (Pereira et al., 2014) and CB1Rs identified in both regions (Eggan et al., 2008; McLeod et al., 2008).

Abbreviations: PolyIC, polyriboinosinic:polyribocytidylic acid; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; mPFC, medial prefrontal cortex; oPFC, orbital prefrontal cortex; NAcc, nucleus accumbens; PND, post-natal day; ED, embryonic day; MIA, maternal immune activation; CB1R, cannabinoid receptor-type1; ANOVA, analysis of variance; SEM, standard error of the mean; ErbB4, neuregulin-1 receptor; GABA, gamma-amino-butyric acid

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2. Methods

2.1. Animals

Pregnant Wistar rat dams were sourced from the Animal Resource Centre (Perth, Australia). They were kept at a constant temperature of 22 ± 2 °C on a 12–12 h light-dark cycle with lights on at 9am. All animal handling and procedures were carried out in accordance with guidelines established by the Animal Care and Ethics Committee at the Australian Nuclear Science and Technology Organisation (Ethics #244).

The experimental design and treatment protocol has been described previously (Dalton et al., 2012). Briefly, pregnant rats were treated with a single intravenous dose of polyIC (4 mg/kg dissolved in phosphate buffered saline) on embryonic day (ED) 15 and off-spring with intraperitoneal HU210 (100 μg/kg dissolved in a vehicle solution of Tween80:dimethyl sulfoxide:saline (1:1:98)) at post natal day (PND) 35 for 14 days. All injections were administered at a volume of 1 mL/kg. Rats were euthanized at PND 65 corresponding to early adulthood (Andersen, 2003; Spear, 2000) (Fig. 1). Rats were divided into four groups: vehicle/vehicle ($n=5$) (control group), polyIC/vehicle ($n=5$) vehicle/HU210 ($n=4$), and polyIC/HU210 ("two-hit") ($n=5$).

2.2. Brain dissections

Whole brains were removed, rapidly frozen in liquid nitrogen and stored at −80 °C. Using a rat brain slice matrix with 1.0 mm coronal slice intervals, two 2 mm thick coronal slices were obtained (coronal section-1 bregma 4.70-2.70, coronal section-2 bregma 2.70-0.70) (Watson, 2005). Slices were dissected into four areas of interest: medial prefrontal cortex (mPFC) and orbital prefrontal cortex (oPFC) from coronal section-1; and dorsal striatum and nucleus accumbens (NAcc) (ventral striatum) from coronal section-2.

2.3. Western blotting

Protein levels of EGFR, ErbB4 and PI3K p110δ were measured by Western blot using standard procedures (Pereira et al., 2012). Antibodies recognising EGFR (1:1000, 175 kDa, Cell Signalling Technology, Cambridge, USA), ErbB4 (1:1000, 180 kDa, Cell Signalling Technology), PI3K p110δ (1:2000, 119 kDa, Abcam, Cambridge, USA) and GAPDH as a loading control (1:500, 38 kDa, Millipore, Massachusetts, USA) were used.

2.4. Real-time quantitative PCR (qPCR)

Total RNA was isolated from 15 to 38 mg of frozen brain tissue from the four regions using TRizol reagent (Invitrogen, California, USA) and RNeasy Kit (QIAGEN, California, USA) according to the manufacturers' instructions. For cDNA synthesis, 2 μg RNA was reverse transcribed using Superscript First-Strand Synthesis Kit according to manufacturer's instructions (Invitrogen).

qPCR was performed in optical 384-well plates with a ViiA7 Fast-System (Applied Biosystems, California, USA) and undertaken in 10 μl reaction volumes containing 5 μl Taqman master mix, 0.4 M forward and reverse primers, and 10 ng cDNA dissolved in nuclease-free water. PCR conditions comprised a 10 min pre-incubation at 95 °C, followed by 40 cycles of 15 s at 95 °C and 60s at 60 °C. Fluorescence was measured at 522 nm wavelength at each annealing step. Predesigned Taqman gene expression assays (Applied Biosystems) targeting EGFR (AssayID: Rn00580398_m1), ErbB4 (AssayID: Rn00572447_m1) and PI3K p110δ (Assay ID: Rn01516709_m1) were used to measure the mRNA levels of the corresponding genes. Three housekeeping genes were also assayed: 18 s (AssayID: Hs99999901_s1), GAPDH (AssayID: Rn99999916_s1) and YWAZ (AssayID: Rn00755071_m1). Reactions were performed in triplicate and normalised to the geometric mean of the housekeeper genes.

2.5. Statistical analysis

Data were pooled with each treatment group and presented as mean ± standard deviation (SD). Differences in protein and gene expression between treatment groups were tested using one-way analysis of variance (ANOVA) and two-way ANOVA in each brain region and corrected by post-hoc Tukey's multiple comparison test to discriminate differences between groups with $P<0.05$ set as statistically significant. Statistical analysis was performed using Prism5.01 (GraphPad).

3. Results

One-way ANOVA for EGFR protein levels showed there was an overall effect of treatment in the NAcc ($F_{3,12}=5.18$, $P=0.016$) and in the dorsal striatum ($F_{3,12}=8.54$, $P=0.003$) with no changes seen in the mPFC ($F_{3,14}=0.58$, $P=0.64$) or oPFC ($F_{3,15}=0.37$, $P=0.77$) (Table 1). Rats exposed to both MIA and adolescent cannabis had significantly increased levels of EGFR protein in the NAcc compared to controls and polyI:C treated animals ($P=0.03$ and $P=0.04$ respectively, post-hoc Tukey's test, Fig. 2). "Two-hit" rats also had significantly increased levels of EGFR protein in the dorsal striatum compared to controls, polyI:C only and HU210 only treated animals ($P=0.01$, $P=0.004$ and $P=0.01$ respectively, post-hoc Tukey's test, Fig. 2).

Two-way ANOVA for EGFR levels with factors of treatment and brain regions indicated significant main effects of treatment ($F_{3,56}=3.75$, $P=0.016$) and brain region ($F_{3,56}=2.88$, $P=0.044$) however there was no interaction of treatment and brain region ($F_{9,56}=0.87$, $P=0.56$). There were no changes in protein or mRNA expression levels for ErbB4 and PI3K p110δ in any of the other four brain regions examined (Supplementary Figs. 1 and 2, Supplementary Table 1).

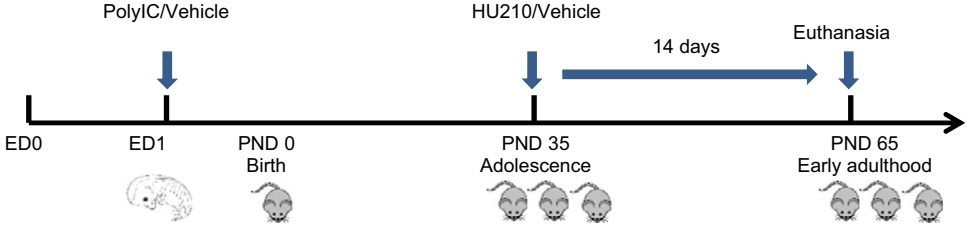

Fig. 1. Experimental design: PolyIC or vehicle was administered to pregnant dams on ED15, whilst administration of the cannabinoid receptor agonist (HU210) or vehicle occurred in adolescence on PND 35 for 14 days, and rats euthanized on PND 65 corresponding to young adulthood.