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Original article

Involvement of vascular endothelial growth factor (VEGF) and mitogen-activated protein kinases (MAPK) in the mechanism of neuroleptic drugs

Marta Jóźwiak-Bębenista[a,*], Magdalena Jasińska-Stroschein[b], Edward Kowalczyk[a]

[a] Department of Pharmacology and Toxicology, The Interfaculty Chair of Basic and Clinical Pharmacology and Toxicology, Medical University of Lodz, Łódź, Poland

[b] Department of Biopharmacy, Medical University of Lodz, Łódź, Poland

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ABSTRACT

Background: Recent evidence suggests that the mitogen activated protein kinase (MAPK)-associated signaling pathway in the frontal cortical areas demonstrates abnormal activity in cases of schizophrenia. Moreover, schizophrenia patients often display alterations in the regional cellular energy metabolism and blood flow of the brain; these are shown to parallel changes in angiogenesis primarily mediated by vascular endothelial growth factor (VEGF).

Methods: The present study examines the differential effects of time-dependent treatment with haloperidol, olanzapine and amisulpride (20 μM) on VEGF and MAPK mRNA expression and VEGF level, using the T98 cell line as an example of nerve cells. For the purposes of comparison, the effect of neuroprotective pituitary adenylate cyclase-activating polypeptide (PACAP) on the expression of VEGF mRNA and secretion were also evaluated in this cell model.

Results: RT-PCR analysis revealed that all the tested neuroleptics increased VEGF mRNA expression after 72-h incubation; however, only haloperidol and olanzapine also increased the level of VEGF detected by ELISA, and they demonstrated significantly stronger effects than PACAP. Haloperidol and olanzapine, but not amisulpride, decreased MAPK14 mRNA expression in T98G cells after 72-h incubation.

Conclusion: The obtained results suggest that haloperidol and olanzapine can trigger the MAPK and VEGF signaling pathway, which may contribute to their neuroprotective mechanism of action.

Introduction

The etiology of schizophrenia remains unclear. It has been reported that the various positive and negative symptom dimensions observed in subjects with schizophrenia are associated with complex genetic and environmental interactions that impair neurodevelopmental processes. Patients with schizophrenia have demonstrated abnormalities in the mitogen activated protein kinase (MAPK)-associated signaling pathway in the frontal cortical areas [1]. Dysfunctions have also been noted in intracellular signaling, neural plasticity, the energy metabolism of certain cells and the overall blood flow around the brain [2]. Following the remodelling of cellular metabolic activity, the demand for oxygen and glucose will change resulting in changes in blood flow to compensate; this will take place by the induction of acute vasodilatation and the formation of new capillaries from pre-existing blood vessels, known as overtime angiogenesis [3,4].

One of the major factors involved in the process of angiogenesis is vascular endothelial growth factor (VEGF). This potent angiogenetic factor is involved in the regulation of blood flow and is characterized by neurotrophic and neuroprotective properties [5]. A defect in VEGF gene expression would therefore be expected to influence the pathophysiology of schizophrenia. Postmortem studies of schizophrenia subjects show a significant reduction in VEGF mRNA expression in the dorsolateral prefrontal cortex and decrease in VEGF receptor 2 (VEGFR2) levels in the prefrontal cortex [4,6]. In addition, plasma VEGF levels were found to be significantly lower than in healthy controls [7]. Thus, the potential of VEGF-mediated influence to increase both angiogenesis and neurogenesis might represent a new approach to treating such

* Corresponding author at: Department of Pharmacology and Toxicology, The Interfaculty Chair of Basic and Clinical Pharmacology and Toxicology, Medical University of Lodz, Zeligowskiego 7/9, Lodz, PL 90-752, Poland.
E-mail address: marta.jozwiak-bebenista@umed.lodz.pl (M. Jóźwiak-Bębenista)

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psychiatric disorders as schizophrenia. Nevertheless, it is first necessary to understand how existing neuroleptic drugs normalize brain blood flow by modulating the angiogenic action of VEGF.

Although the mechanisms by which existing antipsychotic drugs act on schizophrenia are complex and poorly understood [8–10], it is thought that they modulate monoaminergic neurotransmission, mainly through dopaminergic pathways [11]. Recent studies have suggested that antipsychotics have a neurotrophic factor and neuropeptide-mediated neuroprotective mechanism of action [12]. Moreover, some studies suggest that antipsychotics probably increase or decrease neuronal metabolic activity through their neurotransmitter blockade, which may alter blood flow. Some previous research has been carried out on the relationship between antipsychotics and VEGF levels in both schizophrenia patients and animal models of schizophrenia and the effects associated with first generation (FGAs) and second generation antipsychotic drugs (SGAs) have been found to differ [7,13].

Although angiogenesis is best characterized in non-neural tissues, it also occurs in the CNS; however, little information is present on the neuroleptic modulation of VEGF at the mRNA and protein levels in different CNS cell types, apart from in homogenates. VEGF and VEGF receptors are expressed on nerve cells, as well as numerous non-endothelial cells [14]. Astrocytes help promote endothelial cell proliferation and activation by producing VEGF; this is used to control angiogenesis and to assist blood flow regulation [13].

The aim of the current paper is to compare the effect of haloperidol (FGAs), olanzapine and amisulpride (SGAs) on the expression of VEGF mRNA and protein from astrocyte-like model T98G glioblastoma cells. It also examines whether the tested drugs also have an influence on MAPK mRNA expression. T98G human cells were used in the study. They are similar to primary astrocytes and are commonly used in astrocyte research [15–17]. Pituitary adenylate cyclase-activating polypeptide (PACAP), was used as a reference – this is a neuropeptide with known pleiotropic properties and strong neuroprotective potential [18,19]. For the purposes of comparison, the effect of exogenous PACAP on the expression of VEGF mRNA and secretion were also evaluated in this cell model.

Materials and methods

Cell cultures

The human T98G glioblastoma cell line was purchased from the American Type Culture Collection (Rockville, MD, USA). The cells were grown in 25 cm^3 flasks in Advanced Minimum Essential Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 2 mmol/l L-glutamine (Sigma-Aldrich) and an antibiotic solution: 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen), in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Cells were harvested every third day in 0.25% trypsin-EDTA solution (Invitrogen). For the gene expression assay, T98G cells were plated onto six-well plates (Nunc) at a density of 3×10^5 cells per well. For the VEGF enzyme-linked immunosorbent assay (ELISA), the cells were seeded onto 24-well plates, at a density of 1×10^5 cells per well. For the cell viability assay, cells were seeded (50 000 cells/well) into 96-well plates.

Drug and peptide treatments

T98G cells were exposed to the neuroleptic drugs: haloperidol, olanzapine, amisulpride (Sigma Chemical Co., St. Louis, MO, USA), and to the peptide PACAP38 (H-8430, Bachem) and incubated for 24, 72 and 96 h. All the neuroleptic drugs were dissolved in dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO, USA) followed by a preparation of working concentrations in an appropriate medium. Control samples were treated with medium incorporating DMSO in amounts corresponding to the concentration of the used drugs. As PACAP38 was dissolved in water, the control group consisted of medium without the peptide.

RT-PCR analysis

Gene expression was tested by real-time quantitative polymerase chain reaction (RT-qPCR).

The method was performed according to the procedure described in detail in our previous paper [12]. Briefly, total RNA extraction and cDNA were performed according to the manufacturer's instructions. Gene expression was measured using the TaqMan kit. Specific pre-made TaqMan assays were used in this study: vascular endothelial growth factor (VEGF, Hs73793), mitogen-activated protein kinase (MAPK14, Hs485233) and beta actin (ACTB, Hs01060665_g1) as the endogenous control. TaqMan PCR assays were performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems) in FastGene Fast 96-well PCR plates (Nippon Genetics Europe GmbH). The following thermal cycling specifications were performed: 20 s at 95 °C and 40 cycles each for 3 s at 95 °C and 30 s at 60 °C. Real-time PCR data was analyzed using the $2^{-\Delta\Delta Ct}$ method [20].

ELISA assay

VEGF protein levels in the cell-conditioned media were determined using a VEGF ELISA kit, according to the manufacturer's instructions (R&D System, Abingdon, UK). Data is given as pg/ml protein.

MTT conversion

T98G cell viability was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich Chemical Co. Ltd; St. Louis, USA) conversion method. The treated cells were incubated for 72 and 96 h with antipsychotics and without tested drugs (control group). After incubation, 50 μl MTT (1 mg/ml, Sigma) was added and the plates were incubated at 37 °C for 3 h. At the end of the experiment, the cells were exposed to 100 μl dimethyl sulphoxide, which enabled the release of the blue reaction product: formazan. The absorbance at 570 nm was read on a microplate reader and results were expressed as a percentage of the absorbance measured in control cells.

Data analysis

All analyses were performed using Statistica 13.1 Software (StatSoft, Tulsa, OK, USA). Data was presented as mean ± standard error of the mean (SEM) values. The variables were analyzed using one-way or two-way analysis of variance (ANOVA) for repeated measures (time of incubation) followed by the *post-hoc* Student-Newman-Keul's test. These analyses allowed statistically significant alterations in the mRNA and protein levels of VEGF and MAPK to be identified as functions of treatment and time of incubation. If necessary, the data was transformed to meet the assumptions of linearity and homogeneity of variance. A two-tailed p-value less than 0.05 was regarded as statistically significant.

Results

Effects of neuroleptics on VEGF expression in the T98G cell line

Three antipsychotic drugs with different mechanisms of action were chosen: one FGA, represented by haloperidol, and two SGAs,