Pharmacology & Pharmacy, **2013, 4, 443-446**
http://dx.doi.org/10.4236/pp.2013.45063 Published Online August 2013 (http://www.scirp.org/journal/pp)

D_1 and TrkB Receptors Take Charge of the Molecular Antidepressant Action in Cultured Astroglial Cells

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Received May 20th, 2013; revised June 27th, 2013; accepted July 14th, 2013

ABSTRACT

In psychopharmacology of depression, we observe two ways of research. One group is focused on catecholamines action. Second one fixes attention on neuronal morphogenesis and synaptic plasticity. The intimate connection of astrocytes, neurons and synaptic endings determines glial participation in neural homeostasis. Consequently this situation enlarges the role of astrocytes in the CNS synaptic plasticity. Brain Derived Neurotrophic factor and its receptor TrkB suppose to coordinate both of the above mentioning signaling pathways in depression disturbances. In our experiment, we have exploited striatal tissue because in our opinion this structure is misjudged in pathophysiology of depression alas; Several hypothesis proposed striatum as important in future intention activity structure. RT-PCR analysis was used to determine D_1, BDNF and TrkB mRNA expression in cultured striatal astroglial cells. Administration of three representative antidepressants (ADs) like amitriptyline, moclobemide and sertraline to astroglial culture medium increase the D_1, BDNF/TrkB mRNA expression. Our previous study showed that the stimulation of cAMP to CREB pathway after D_1 receptors excitation constituted a common response to ADs. The present results signify that D_1, BDNF/TrkB link which is next neural track (after cAMP/PKA) involved in the CNS adaptation to external conditions altered by chronic ADs treatment. Moreover, the striatum tissue appears to be important formation which takes an active part in antidepressant action thus essential in depression disorder etiology.

Keywords: BDNF; D_1 Receptors; Neurotrophins; TrkB Receptors

1. Introduction

Depression is not only life-threating illness (thoughts and suicides attempts) but also serious social problem (loss of interest in daily activities) and economic difficulty (one of the main causes of long-term work absenteeism). Despite dynamic progress in pharmacology and neuropsychology and synthesis of new generations of antidepressant drugs, pathogenesis of depression, is still unknown.

Up until now, there has been also not much success in univocal establishing of the type of neurochemical transmission responsible for the mechanism of multidirectional action of this structurally diverse group of drugs. Dysfunction of the serotonergic and noradrenergic system and long term brain adaptation process as an important aspect of ADs action seem to be the best documented so far as catecholaminergic supposition of depression.

Right now, major of experiments focused on neuronal morphogenesis, neurodegenerative processes and neuronplastic changes in the brain as helpful approaches to clarify etiology of depression and ADs action [1].

Evidences from animal studies support thesis that brain derived neurotrophic factor, member of the neurotrophin family, is a very important protein which regulates neuron cell life activity through membrane receptors TrkB [1,2]. Next, it is well known that BDNF/TrkB-stimulated signaling is necessary for morphogenesis and neuronal plasticity involved in the pathophysiology of depression [2-5].

Previous experiments conducted in our laboratory have confirmed at the molecular level the contribution of dopaminergic neurotransmission by D_1 receptors→adenylyl-cyclase→cAMP→CREB signaling to the mechanism of action of antidepressant drugs [6-8].

The aim of this study was to establish type of changes in BDNF/TrkB mRNA expression in glial cultures after chronically administered antidepressants acting via different mechanisms [5,9,10].

To be able to directly compare the results we chose the same representatives of ADs as there tested for previous study like: amitriptyline, moclobemide and sertraline.

2. Materials and Methods

2.1. Cell Culture

Striatal astroglial cells were isolated from 1-day-old Wistar rats and cultured according to the method of Hertz [11]. Astrocytes were grown on 35 × 10 mm Falcon dishes covered with poly-D-lysine. The culture medium initially contained 20% FBS was replaced by 10% FBS after 4 days. The culture was changed twice a week at about 14 days. The cells were further cultured for next 2 weeks. In addition cultures were treated with amitriptyline or moclobemide, or sertraline at 10 mM concentration with every 2 days medium-mix change. Immunocytochemical analysis of cultures was shown 90% - 95% GFAP-positive cells.

Ten following experiments for three different ADs respectively were done.

2.2. RNA Isolation

Total RNA was isolated from 10 cell dishes (35 × 10 mm) by Chomczynski [12] extraction method with the TRIzol reagent. Isolated and purified RNA was dissolved in nuclease-free water (Promega) quantified by determining absorbance at 240 and 260 nm, preserving proportion 1OD 260 = 40 mg/ml RNA and stored at −70˚C before use. The OD_{260}/OD_{280} ratio of the isolated samples ranged 1.8 - 2.0, indicating that the material was not contaminated by DNA. Purity testing confirmed proper choice of procedure and reagents for isolation. Starters for RT-PCR were designed using Primer 3 software, from genBank details. Only TrkB starters nucleotide sequence is taken from an A. Bahi, paper [13].

Compositions of starter nucleotides are presented in the **Table 1**.

2.3. Reverse Transcriptase Polymerase Chain Reaction

RT-PCR analysis was used to determine expression of D_1, BDNF/TrkB receptors mRNA in cultured striatal astrocytes exposed to different antidepressant drugs. The Reverse Transcription PCR procedure was performed using ACCESS Promega System with AMV Reverse Transcriptase and Tfl polymerase, in Eppendorf thermocycler, according to the following sequence: reverse transcriptt ion 65˚C 30 min, PCR amplification: previous denaturetion 94˚C, 2 min; denaturation 94˚C, 30 sec, annealing 63˚C, 30 sec, extension 70˚C 30 sec, final extension 70˚C 7 min, soak 4˚C.

Reverse transcription temperature was optimized for each gene around downstream starter hybridization temperature while annealing temperature was optimized depending on upstream starter hybridization temperature and quality of products obtained in pilot experiments.

RT-PCR products were separated by electrophoresis on 2% LMP agarose for separation of short nucleotides (Promega) labeled with ethidium bromide (1 mg/ml). Aliquots of RT-PCR product (10 ml) were collected and 3 ml of loading solution for electrophoresis (40% sucrose, 0.25% bromophenol blue) were added to each sample and loaded in slots of the gel. Electrophoresis was developed in TAE 1x buffer (pH8.0), and run parallel to DNA marker (pUC19/*Msp*I) **Figure 1**.

2.4. Statistical Analysis of Results

Images of the obtained electrophoretic separations were stored using BioProfile E software (France) for data archiving. Densitometric measurements of RT-PCR products after electrophoretic separation were performed using a semiquantitive method *vs*. data obtained for constitutive gene using LabWorks software (UVP, UK). as shown in **Figure 1**. All data are represented as the means ± SEM of the mean relative optical density (ROD), after background subtractions, which were subjected to statistical analysis using one-way ANOVA followed by Dunnett's test for specific comparisons [14]. Significance was established at a level of $p < 0.05$ and $p < 0.01$.

3. Results

RT-PCR (**Figure 2**) analysis present increase of D_1 and BDNF/TrkB mRNA expression in cultured striatal astroglial cells exposed to long term administration of different (in pharmacologic proceedings) ADs to culture medium (amitriptyline, moclobemide, sertraline).

We have shown previously [6] that chronic use of ADs

Table 1. Compositions of starter nucleotides for RT-PCR procedure.

	5' Sense primers 3'	5' Antisense primers 3'	Products
BDNF	AgC CTC CTC TgC TCT TTC TgC Tgg	CTT TTg TCT ATg CCC CTg CAg CCT T	298 bp
TrkB	CCT CgT Tgg AgA AgA TCA Ag	CgT ggT ACT CCg TgT gAT gT	221 bp
D_1	ATC TCA gCC TTg gAg Agg	ggC ACC ATA CAg TTc gAg	206 bp
GAPDH	gTg AC ggA TTT ggC CgT ATC gg	ATC ACg CCA CAg CTT TCC AgA gg	573 bp

All RT-PCR assays were performed in triplicate with positive and negative control.

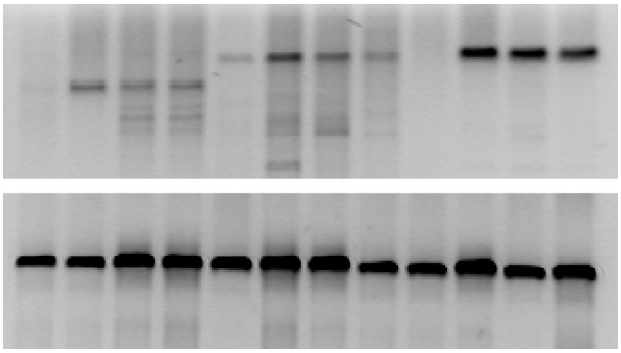

Figure 1. Typical photograph of gel pattern showing BDNF/ TrkB, D_1 and GAPDH bands.

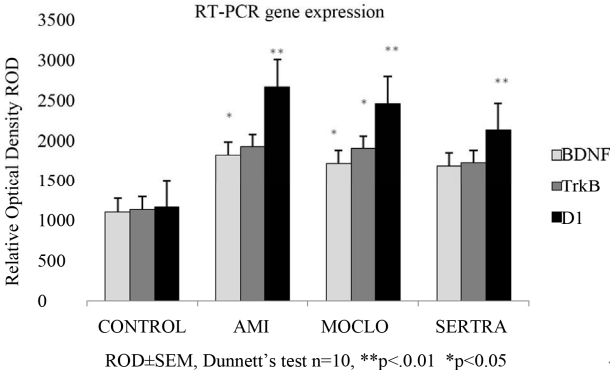

Figure 2. Semi-Quantitative analysis of BDNF/TrkB and D_1 levels by RT-PCR.

increases expression of dopamine D_1 family receptors in both striatal and nucleus accumbens neurons. The present study was designed to investigate chronic effects of ADs on cultured astrocytes dopamine receptors. Our study showed next time that used antidepressants significantly increase dopamine D_1 receptor gene expression.

4. Discussion

Results of the present work indicate that chronic ADs treatment increases BDNF/TrkB mRNA expression in striatal astroglial cells. They are in good according with our reports that cAMP→CREB→BDNF pathway stimulation is a common response to ADs with different initial mechanism of action [15,16]. In our opinion BDNF and its receptor TrkB control and coordinate synaptic changes in depression disturbances and brain reaction to external ADs action stimulus as well. Another important aspect of BDNF is its involvement in the cross-talk between different signaling mechanism: biological and molecular. In addition we next time observe high expression of dopamine D_1 type receptor. In light of previews studies conducted in our laboratory, this observation provides next support to the hypothesis implicating DA in the mechanism of ADs [6,8,17].

The obtained results suggest that ADs, stimulate BDNF/TrkB after D_1 mRNA expression as important link in the CNS adaptation to internal and external conditions. Sequence of events in neuronal cell could be depicted as follows. Chronic ADs treatment increases dopamine level in synaptic space. Dopamine D_1 receptors become strongly activated and, via Gs protein, stimulate adenylylcyclase indispensable for ATP transformation into cAMP inside the stimulated neuronal cell. The signal elicited by cAMP activation is transmitted by the cellular kinase system to the nucleus, with CREB activation. This transcription factor stimulates expression of CRE-sequence-genes that recognize cAMP/PKA pathway. Next CREB activates BDNF through TrkB receptors mRNA expression. In this light BDNF/TrkB signaling becomes a junction for catecholamines transmission and neuronal plasticity activation amplifying antidepressant action and future clinical efficacy.

5. Conclusion

Our result presents molecule evidence for functional role of D_1, BDNF/TrkB link action in long term CNS adaptation. These findings signify that alteration in neurotrophins' family may play key role in pathophysiology of mood disorder. We suppose in addition that our results may be useful to select narrow spectrum of gene markers important in depression illness to potential phamacogenetic studies of the future new antidepressants.

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