IJPT
1735-2657/07/61-67-70
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 6:67-70, 2007

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RESEARCH ARTICLE

Sodium Orthovanadate Treatment Reverses Protracted Methionine Administration Induced Schizophrenia Like Behavior in Rats

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Received November 11, 2006; Accepted May 20, 2007

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Suppression of Akt (Protein kinase B) has been implicated in schizophrenia, the effect of which has been documented to be reversed by tyrosine phosphatase inhibition. Present study has been designed to study the effect of sodium orthovanadate, a tyrosine phosphatase inhibitor, on protracted methionine administration induced schizophrenia-like behavior in rats. Schizophrenia-like behavior was assessed in number of circuits of cage (locomotor activity), number of climbs of sides (climbing), number of rears, face washing, scratching and chewing (stereotypy). Methionine administration (1.7g/kg/d, p.o.) for a period of 30 days elicited schizophrenia-like behavior in rats as assessed in terms of the measures of locomotor activity, climbing and stereotypy. However, sodium orthovanadate (15, 30 & 60 mg/kg, i.p.) coadministration from day 15 to day 30 markedly reduced this methionine induced increase in locomotor activity (from 40 ± 5.45 to 22 ± 2.82), climbing (from 57 ± 4.05 to 22 ± 2.12) as well as stereotypy (from 159 ± 6.9 to 59 ± 3.9). Sodium orthovanadate treatment induced reversal of protracted methionine administration induced schizophrenia like behavior in rats reflecting that inhibition of tyrosine phosphatase might be a useful approach in the therapeutics of schizophrenia.

Keywords: *Methionine, Schizophrenia, Sodium orthovanadate, Tyrosine phosphatase*

Schizophrenia is a psychotic disorder marked by unrealistic, irrational behavior and delusions [1,2]. Although the etiology behind the disease is not completely understood, various studies have indicated that it is a multifactorial disorder characterized by the contribution of multiple susceptibility genes that act in coherence with epigenetic processes and environmental factors [3]. At genetic level, hypermethylation of the reelin promoter down-regulates reelin expression and contributes to the pathophysiology of schizophrenia [4]. Local modulation of protein kinase B/Akt mediates reelin-PI3K (Phosphoinositide-3-kinase) signaling in neuronal growth [15]. Reelin has a role in regulating the event-related increase of protein synthesis mediated by the dendritic translation of cytosolic mRNAs [5-8]. Consequently, the down-regulation of reelin expression in the brain of schizophrenic patients mediates the down-regulation of pyramidal neuron dendritic branching & spine expression, differentiation in the wrong locations and thus in the neuropil hypoplasticity typical of schizophrenia [9-11]. Local modulation of protein kinase B/Akt mediates reelin-PI3K signaling in neuronal growth [12]. Reelin stimulates Disabled 1 (Dab 1) protein tyrosine phosphorylation in cortical neurons thus causing its effects [13,14]. Protein tyrosine phosphatase (PTPase), an enzyme that regulates the tyrosine phosphorylation has been shown to be expressed within dopaminoceptive neurons of the basal ganglia and related structures [16]. Inhibition of PTPase has been documented to activate the PI3K-Akt pathway [17-21]. The present study has been designed to evaluate the effect of sodium orthovanadate, a PTPase inhibitor, on protracted methionine administration induced schizophrenia-like behavior in rats.

MATERIALS AND METHODS

Age matched male sprague-dawley rats without any overt signs of abnormally increased locomotion, climbing & stereotypy, weighing 250 ± 15g were obtained from Indian Veterinary Research Institute, Izatnagar, India, maintained on standard laboratory diet (Kisan

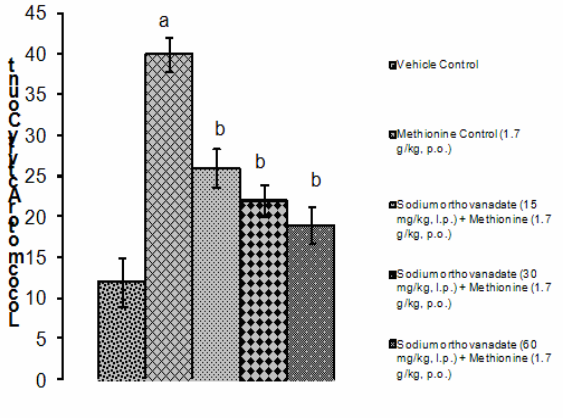

Fig. 1. Effect of sodium orthovanadate on methionine-induced increase in locomotor activity in rats. [Responses are expressed as number of circuits in the observation chamber. Values are mean ± SEM (n=10). Statistical analysis was done using one way ANOVA followed by post hoc, Tukey's multiple range test. a=p<0.05 vs. vehicle control; b=p<0.05 vs. methionine control.]

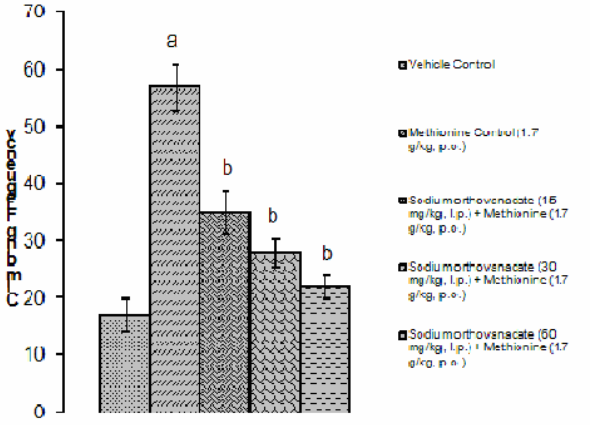

Fig. 2. Effect of sodium orthovanadate on methionine-induced increase in climbing activity in rats. [Responses are expressed as the frequency of climbing activity. Values are mean ± SEM (n=10). Statistical analysis was done using one way ANOVA followed by post hoc, Tukey's multiple range test. a=p<0.05 vs. vehicle control; b=p<0.05 vs. methionine control.]

Feeds Ltd., Mumbai, India) and had free access to tap water. They were housed in the departmental animal house and were exposed to 12 hour cycle of light and dark. The animal experiments were carried out as per the guidelines of institutional animal ethical committee and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environmental and Forests, Government of India (Reg. No.- 107/1999/CPCSEA). The laboratory temperature was maintained at 25 ± 3°.

Drugs and chemicals

Methionine was obtained from SD-fine chemicals Pvt. Ltd., India and sodium orthovanadate was obtained from Sigma-Aldrich Chemicals Pvt. Ltd., St. Louis, USA. Methionine and sodium orthovanadate were suspended in 0.5% w/v solution of carboxymethylcellulose (CMC). The animals were acclimatized to the laboratory conditions for 24 hours before drug administration. The doses and schedules of drug administration employed in the present study were based on the well established data of certain cardiovascular models established in our laboratory (viz. Endothelial Dysfunction & Cardiac Hypertrophy) and on the pilot studies for this very study on schizophrenia-like behavior using the employed doses of methionine as well as sodium orthovanadate in rats.

Experimental protocol In the present study a total of five groups were employed and each group comprised of 10 animals.

Group I (Vehicle treated control group): Rats were administered vehicle (0.5% w/v CMC, 4 ml kg-1, p.o.) daily for a period of 30 days.

Group II (Methionine treated schizophrenic control group): Rats were administered methionine (1.7g/kg, p.o.) daily for a period of 30 days.

Group III (Low dose Sodium orthovanadate + Methionine treated schizophrenic group): Rats were administered methionine (1.7g/kg, p.o.) daily for a period of 30 days and sodium orthovanadate (15mg/kg, i.p.) once daily from day 15 to day 30 of the dosing regimen.

Group IV (Intermediate dose Sodium orthovanadate + Methionine treated schizophrenic group): Rats were administered methionine (1.7g/kg, p.o.) daily for a period of 30 days and sodium orthovanadate (30mg/kg, i.p.) once daily from day 15 to day 30 of the dosing regimen.

Group V (High dose Sodium orthovanadate + Methionine treated schizophrenic group): Rats were administered methionine (1.7g/kg, p.o.) daily for a period of 30 days and sodium orthovanadate (60mg/kg, i.p.) once daily from day 15 to day 30 of the dosing regimen.

Behavioral Observations

For assessment of behaviors each rat was observed for a period of 10 minutes by the same trained observer who manually quantified behaviors in a semi-sound

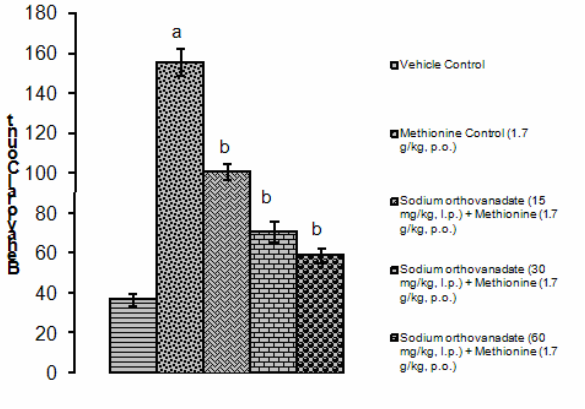

Fig. 3. Effect of sodium orthovanadate on methionine-induced stereotypy in rats. [Responses are expressed as the total of the frequencies of rears, face washing, scratching and chewing behaviors. Values are mean ± SEM (n=10). Statistical analysis was done using one way ANOVA followed by post hoc, Tukey's multiple range test. a=p<0.05 vs. vehicle control; b=p<0.05 vs. methionine control.]

proof laboratory.

Number of circuits made by the rat in a transparent Perspex observation chamber was observed for 10 minutes and recorded as an assessment of the locomotor activity.

Number of climbs also made by the rat in a transparent Perspex observation chamber of sides was observed to quantify climbing behavior.

Stereotypy was quantified by recording number of rears, face washing, scratching and chewing behaviors based on the observations made after placing the rat in the observation chamber.

These behavioral parameters were studied manually as a mean of schizophrenia-like behavior according to the procedure described by Ljungberg & Ungerstedt, 1985 and Newson *et al.,* 2006 [22, 23]. Increase in locomotor activity, climbing behavior (number of climbings) and stereotypy was taken as an index of schizophrenia like behavior.

Statistical Analysis

The results were expressed as the mean ± standard error of mean (SEM) frequency of observations displayed by the rat. Statistical analysis for the results was done using one way ANOVA followed by post hoc, Tukey's multiple range tests. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of methionine and combination of methionine & sodium orthovanadate on locomotor activity of rats.

Methionine administration (1.7g/kg, p.o.) for a period of 30 days significantly increased locomotor activity as compared to the vehicle treated group. However, sodium orthovanadate (15mg/kg, i.p., 30mg/kg, i.p. & 60mg/kg, i.p.) co-administration from day 15 to day 30 of methionine dosing significantly and dose dependently reversed the effect of methionine on locomotor activity (Fig 1).

Effect of methionine and combination of methionine & sodium orthovanadate on climbing activity of rats.

Methionine administration (1.7g/kg, p.o.) for a period of 30 days significantly increased climbing activity as compared to the vehicle treated group. However, sodium orthovanadate (15mg/kg, i.p., 30mg/kg, i.p. & 60mg/kg, i.p.) co-administration from day 15 to day 30 of methionine dosing significantly and dose dependently reduced the effect of methionine on climbing activity (Fig 2).

Effect of methionine and combination of methionine & sodium orthovanadate on Stereotypy in rats.

Methionine administration (1.7g/kg, p.o.) for a period of 30 days elicited a marked increase in the measure of stereotypy as assessed in terms of number of rears, face washing, scratching and chewing behaviors compared to the vehicle treated control group. However, sodium orthovanadate (15mg/kg, i.p., 30mg/kg, i.p. & 60mg/kg, i.p.) co-administration from day 15 to day 30 of methionine dosing significantly and dose dependently attenuated the effect of methionine on stereotypic behavior (Fig 3).

DISCUSSION

An increase in locomotor activity along with elevated climbing behavior and stereotypy mimics schizophrenia-like behavior in animals. Manual observations of these parameters in animals as an index of schizophrenia-like behavior have been reported by studies carried out previously [22, 23]. However, limitations of manual observation cannot be ignored at this point. Protracted (chronic) methionine administration elicited schizophrenia-like behavior in rats as assessed in terms of measures of locomotor activity, climbing and stereotypy [22, 23]. It has been reported that the protracted administration to experimental animals of methionine, a precursor of the methyl donor S-adenosyl-methionine, generates behavioral characteristics of the psychosis in schizophrenia by suppressing reelin mRNA expression [24]. Reelin-induced sites of tyrosyl phosphorylation on Disabled 1 (Dab 1) protein have been shown to be primarily mediating the signaling pathway that control the neuronal positioning that is abnormal in schizophrenia [13, 14]. Thus, this effect of methionine may be attributed to its activity as a precursor of the methyl donor S-adenosyl-methionine and so suppressing reelin mRNA expression due to the hypermethylation of the reelin promoter. Moreover, sodium orthovanadate was observed to dose dependently reverse the schizophrenia like behavior brought forth by methionine administration as assessed in terms of the measures of locomotor activity, climbing and stereotypy. Protein tyrosine phosphatase (PTPase), an enzyme that regulates the tyrosine phosphorylation is expressed within dopaminoceptive neurons of the basal ganglia and related structures [16]. Reelin-mediated phosphatidylinositol 3-kinase signaling in neuronal growth contributes to final neuron positioning in the mammalian brain by local modulation of protein kinase B/Akt [12]. Moreover, inactivation of Akt pathway has been implicated in the sustained dopamine D_2 receptor stimulation mediated behavioral aberrations in schizophrenia [15]. Inhibition of PTPase has been documented to activate the PI3K-Akt pathway [17-21]. Thus the effect of sodium orthovanadate may be ascribed to its indirect facilitation of the suppressed reelin transduction pathway through its PTPase inhibition and consequent activation of PI3K-Akt second messenger system.

On the basis of above discussion, it may be concluded that sodium orthovanadate exerts an inhibitory effect over the methionine induced schizophrenia-like behavior in rats possibly through the facilitation of reelin transduction process through its PTPase inhibition and consequent activation of the Akt system and it may be implicated that inhibition of tyrosine phosphatase

might be a useful approach in the therapeutics of schizophrenia.

ACKNOWLEDGEMENT

Authors are thankful to Dr. A. K. Tiwary, Head, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala, Punjab (India), for his constant encouragement and for providing research facilities.

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