

Clozapine acute administration modified neuronal ATPase activity in spontaneously hypertensive rats

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ABSTRACT

It has been previously reported that the peptide neurotensin (NT) produces an inhibitory effect on Na^+ , K^+ -ATPase (NKA) activity without changes in Mg^{2+} -ATPase activity in Wistar rats. Clozapine i.p. administration, an atypical antipsychotic, 30 min before cortical membrane preparation produced a stimulatory effect at 10 mg/kg clozapine. Taking into account these results, it is of interest to study the effect of neurotensin after clozapine administration on spontaneously hypertensive (SH) and Wistar Kyoto (WKY) rats. Spontaneously hypertensive rat is considered as a model of schizophrenia because this strain develops behavioural deficits in a social context which are attenuated by acute clozapine administration. Experiments conducted in cortical synaptosomal membranes led to a significant increase in NKA activity in both SH and WKY rats after clozapine administration. Neurotensin only produced NKA inhibition in WKY rats, which were previously administered with clozapine. It was observed that this treatment increases the NKA activity values, making it possible to observe the inhibitory effect of neurotensin. But, the increase in NKA activity values were not enough to confirm the inhibitory effect of neurotensin on NKA activity in SH rats. The lack of this neurotensin effect can be explained by the existence of plasma membrane alteration due to hypertensive state, which resulted in modifications of enzymes or receptors inserted into the plasma membrane, and hence the ATPase modulation by the peptide was also modified.

KEYWORDS: clozapine, neurotensin, ATPase activity, spontaneously hypertensive rats.

ABBREVIATIONS

NKA, Na^+ , K^+ -ATPase; SH rat, Spontaneously hypertensive rat; WKY rat, Wistar Kyoto rat.

INTRODUCTION

Na^+ , K^+ -ATPase (NKA) is a plasma membrane enzyme ubiquitous in animal cells, which catalyses the efflux of three Na^+ and influx of two K^+ per ATP hydrolyzed in the maintenance of membrane potential [1]. Structurally NKA is composed of alpha, beta and gamma subunits. Alpha is the catalytic subunit which is composed of $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 4$ isoforms. The isoforms $\alpha 2$ and $\alpha 3$ are found in neurons whereas the $\alpha 1$ and $\alpha 2$ isoforms are localized in glial cells. It should be noted that $\alpha 2$ isoform is expressed in neurons during late gestation and then it is expressed in astrocytes in the adult brain. Beta subunit is required for enzyme assembly and gamma subunit belongs to FXYD proteins whose main function is the regulation of NKA kinetics [2, 3].

NKA is involved in blood pressure regulation because it has been reported that highly conserved cardiac glycoside binding site of the Na^+ , K^+ -ATPase participates in such regulation; specifically it mediates the development and maintenance of ACTH-induced hypertension [4]. Besides, the modulation of NKA activity is crucial for neurotransmission, since natural substances such as peptides (neurotensin) can exert a modulatory effect on this enzyme activity.

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Neurotensin is a tridecapeptide widely distributed in the central nervous system (CNS) where it behaves as a neuromodulator or a neurotransmitter interacting with G protein-coupled receptors termed NTS1 and NTS2 [5]. Previously, it has been reported that neurotensin inhibits synaptosomal membrane Na^+ , K^+ -ATPase activity by the interaction with neurotensin receptors [6, 7] or by a direct action on ouabain site of NKA enzyme [8]. Although neurotensin produces a biphasic effect on blood pressure, which is dependent on infused peptide doses [9], this study was focused on neurotensin as a neuroleptic endogenous agent which modulates dopaminergic pathways through different mechanisms [10, 11]. Deficits in neurotensin neurotransmission have been implicated in the pathophysiology of schizophrenia; thus it has been reported that schizophrenic patients have a 40% decrease in neurotensin receptor population which are located in the entorhinal cortex [12]. Besides, neurotensin levels in cerebrospinal fluid (CSF) are decreased in schizophrenic patients, but they are normalized after antipsychotic therapy [13].

Currently, spontaneously hypertensive (SH) rats are considered as an animal model of schizophrenia [14] because diverse lines of evidence have demonstrated that SH rats present increased behavioral activity and emotionality, hyperlocomotion and have deficits in social interaction that are reversed after acute administration of antipsychotic drugs [15]. Wistar rats based on the expression of spontaneous hypertension have been selected by Okamoto at Kyoto University (1963) to produce a strain of spontaneously hypertensive (SH) rats. At the same time, Wistar Kyoto rats (WKY) with "normal" blood pressure were selected for use as normotensive controls of the SH rats. Clozapine is an atypical antipsychotic agent that mainly blocks 5HT_{2A}/D₄ receptors [16] and modifies the neurotensin effect on NKA activity in accordance with time and dose conditions employed [17].

Therefore, the purpose of this work was to study neurotensin modulation of cortical ATPase activity in SH rats, which were acute administered with clozapine.

MATERIALS AND METHODS

All reagents were of analytical grade. Ouabain, disodium ATP (grade I, prepared by phosphorylation

of adenosine) and neurotensin acetate were from Sigma Chemical Co., St. Louis, MO, USA. Clozapine was given by laboratories Fabra S.R.L., Argentina.

Male Wistar Kyoto (WKY) and spontaneously hypertensive rats (SH) rats were used in this study. All studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA and the Committee on Animal Experimentation (CICUAL), Universidad de Buenos Aires, Argentina.

Drug administration

A lot of six rats were administered intraperitoneally with 18 mg/kg clozapine dissolved in 0.3 mM acetic acid (vehicle) and six rats received vehicle (control). Thirteen minutes later animals were killed by decapitation, skulls opened and cerebral cortices harvested, homogenized and subjected to differential and sucrose gradient centrifugation to obtain synaptosomal membranes.

Enzyme assays

ATPase activity was measured as described by Albers [18]. Synaptosomal membrane fractions were preincubated with 0.20 M Tris-HCl buffer (pH 7.4) or 10^{-6} M neurotensin solution at 37 °C for 10 min. Total ATPase activity was assayed in a medium containing 100 mM NaCl, 20 mM KCl, 3 mM MgCl_2 , 0.20 M Tris-HCl buffer (pH 7.4) and 4 mM ATP during the incubation period. Mg^{2+} -ATPase activity was determined in a similar medium with no added Na^+ and K^+ and containing 1 mM ouabain. The activity resulting from the subtraction of the total ATPase activity minus Mg^{2+} -ATPase corresponds to the activity of Na, K-ATPase. This activity was monitored by colorimetric determination of orthophosphate released [19]. Protein measurement was determined by the method of Lowry [20] using bovine serum albumin as standard.

Statistical analyses

Data are presented as mean values \pm SD of n experiments. To compare groups *inter se* one-way analysis of variance was performed followed by Bonferroni's Multiple Comparison Test. Probability level indicative of statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

ATPase activities were determined in synaptosomal membranes isolated from rat cerebral cortex. NKA and Mg^{2+} -ATPase activities were studied in membranes from WKY and SH rats, animals which were previously administered with vehicle or clozapine, an atypical antipsychotic agent. Besides, neurotensin enzyme modulation was evaluated through *in vitro* experiments.

NKA and Mg^{2+} -ATPase basal activity values were 15.8 and 19.7 $\mu\text{mol Pi} \times \text{mg} \cdot \text{prot}^{-1} \times \text{h}^{-1}$, assayed in WKY rat membranes, whereas in SH rat membranes NKA activity value was 34.6 $\mu\text{mol Pi} \times \text{mg} \cdot \text{prot}^{-1} \times \text{h}^{-1}$ and Mg^{2+} -ATPase activity was 27.4 $\mu\text{mol Pi} \times \text{mg} \cdot \text{prot}^{-1} \times \text{h}^{-1}$. It is worth noting that these results were different from those recorded in Wistar rats, because NKA and Mg^{2+} -ATPase basal activity values are roughly 25.0 $\mu\text{mol Pi} \times \text{mg} \cdot \text{prot}^{-1} \times \text{h}^{-1}$ and 15.6 $\mu\text{mol Pi} \times \text{mg} \cdot \text{prot}^{-1} \times \text{h}^{-1}$, respectively [16]. ATPase total activity values should be at least twice or more than Mg^{2+} -ATPase activity values to probe the existence of ouabain-sensitive activity or NKA activity which is commonly inhibited by diverse substances like steroidal glycosides, vanadate and peptides. Based on this experimental condition neurotensin *in vitro* produce NKA inhibition through the interaction with its receptors [6]. But, when Mg^{2+} -ATPase activity values exceeded total ATPase values, NKA activity values were very low or insignificant. Thus, the enzyme inhibition by selective inhibitors would be impossible and the neurotensin inhibitory effect would not be evident.

Previously, it was reported that clozapine treatment differentially modifies the further effect of neurotensin on NKA activity [16]. Clozapine 18 mg/kg was administered to rats 30 min before membrane preparation since it has been described that acute clozapine administration can reverse deficits in social interaction of SH rats [14]. After acute administration of clozapine, the difference between total ATPase and Mg^{2+} -ATPase activity values was increased, leading to an increase in NKA activity, and neurotensin enzyme inhibition was possible (Fig. 1A, 1B). These facts would indicate that a certain NKA activity value must be reached in order to achieve the modulatory effect of this enzyme activity.

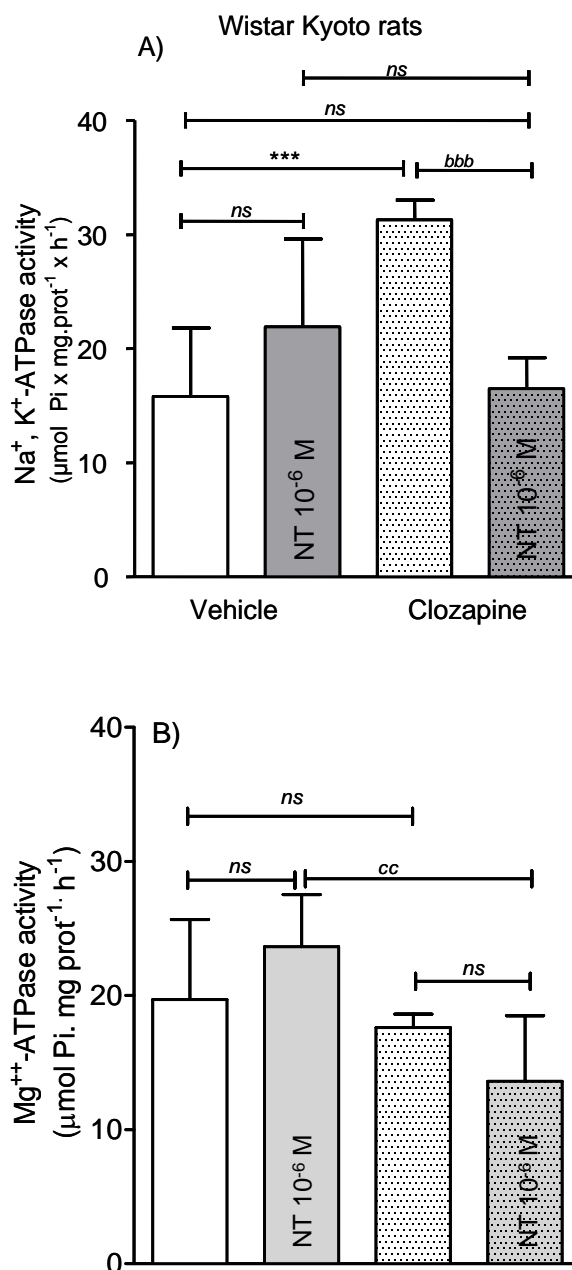


Fig. 1. A) Na^+, K^+ -ATPase and B) Mg^{2+} -ATPase activities. Cortical synaptosomal membranes from Wistar Kyoto rats were preincubated in the absence or presence of neurotensin (NT), 10^{-6} M, and assayed for ATPase activity. Results are expressed in $\mu\text{moles Pi} \times \text{mg} \cdot \text{prot}^{-1} \times \text{h}^{-1}$. SD of 4-8 experiments performed per triplicate is indicated within the bars. One-way analysis of variance followed by *post hoc* Bonferroni test was used, where $***P < 0.001$ corresponds to the control without neurotensin; $bbbP < 0.001$ corresponds to the control in clozapine-administered rats; and $^cP < 0.01$ between treatments in the presence of NT.

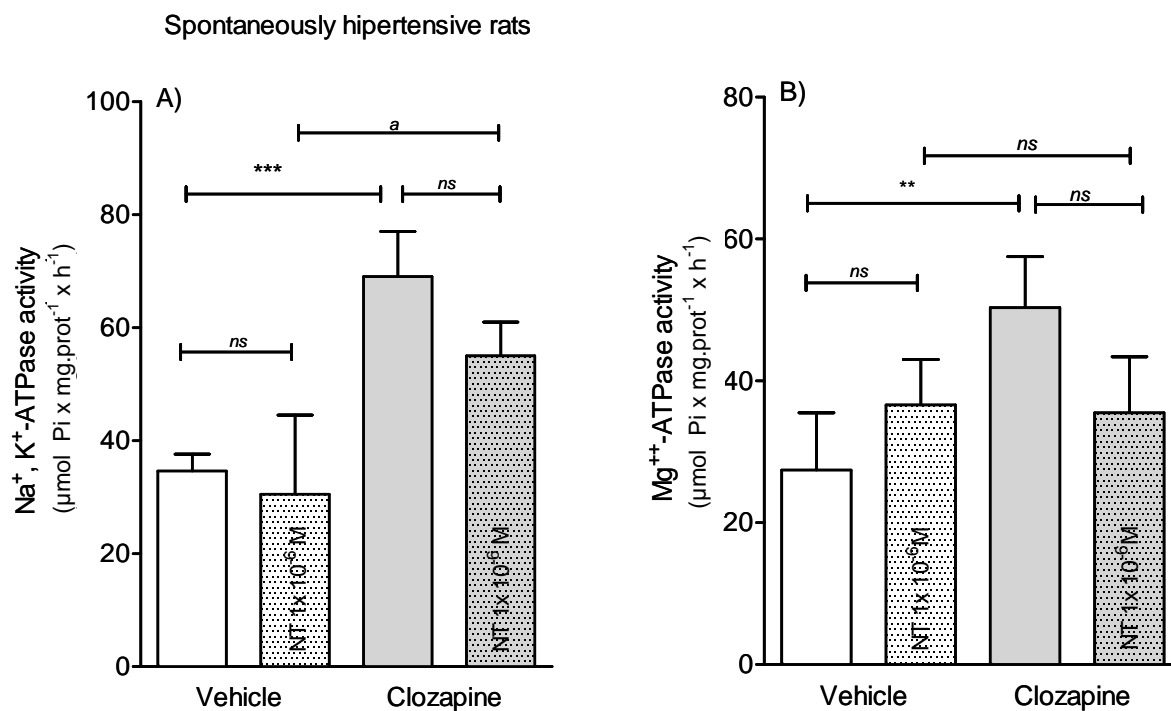


Fig. 2. A) Na⁺, K⁺-ATPase and B) Mg²⁺-ATPase activities. Cortical synaptosomal membranes from spontaneously hypertensive rats were preincubated in the absence or presence of neurotensin (NT), 10⁻⁶ M and assayed for ATPase activity. Results are expressed in µmoles Pi x mg.proton⁻¹ x h⁻¹. SD of 4-8 experiments performed per triplicate is indicated within the bars. One-way analysis of variance followed by *post hoc* Bonferroni test was used, where ***P* < 0.01; ****P* < 0.001 corresponds to the control without NT; and ^a*P* < 0.05 corresponds to the control in the presence of NT.

The increases in mean arterial pressure and heart rate as well as a decrease in baroreflex function indicate physiological changes in SH rats; therefore the modification of NKA activity could be also expected. Herein, NKA basal activity was increased in membranes from clozapine-administered rats, but neurotensin did not produce any inhibitory effect on this enzyme (Fig. 2A, 2B).

These results could be explained by the hypertensive state of SH rats, which lead to membrane damages probably by oxygen free radical production and enzyme activation [21]. Structural abnormalities and dysfunction of mitochondria have been observed in SH rats [22, 23]. Thus, it is very likely that membrane damages due to the hypertensive state may also produce structural and functional modifications in enzymes and receptors linked to the membrane, such as NKA and neurotensin receptors. Since neurotensin effect on the NKA activity depends on the peptide interaction with neurotensin receptors, the structural integrity of its

receptors is an essential condition for the inhibitory effect of neurotensin on NKA activity.

CONCLUSION

Clozapine acute administration resulted in an increase in the NKA basal activity, but the addition of neurotensin did not result in any changes in SH rats which may be due to alterations in plasma membranes caused by the development of the hypertensive state in these animals.

ACKNOWLEDGMENT

Georgina Rodríguez de Lores Arnaiz is a Chief Investigator of Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). Financial support was provided by CONICET and Universidad de Buenos Aires, Argentina.

CONFLICT OF INTEREST STATEMENT

The author declares that there are no conflicts of interest.

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