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Somatostatin Modifies L-NAME Effects on ATPase Activity

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Abstract: **Somatostatin (SST) is a peptide which biological effects are due to the interaction with five receptor subtypes (somatostatin1-5) which can be blocked by the antagonists, such as cyclo-somatostatin (cSSTA). The assay for ATPases was performed in synaptosomal membranes of rat cerebral cortex, basal Na^+, K^+ -ATPase activity was 18.0 ± 5.3 µmol Pi x mg. $prot^{-1}$x h^{-1}, there was not any changes by the presence of 10^{-9}-10^{-6}M SST, but SST 10^{-5}M produced a significant increase in Na^+, K^+-ATPase activity. Besides Mg^{2+}- ATPase activity was not modified by all SST concentrations employed. SST effects on ATPase activity was completely blocked by the previous addition of cSSTA, a somatostatin receptor antagonist, which is suggested that SST receptors could be involved in these effects.**

Na^+, K^+ -ATPase activity was also studied in membranes isolated from adult rats which were administered with vehicle (saline) or L-NAME, a nitric oxide synthase inhibitor. When L-NAME was administered to rats during the postnatal period, it was observed an increase in Na^+, K^+-ATPase activity in the presence of 10^{-6} M SST to membranes prepared in the adulthood of those animals.

L-NAME was also administered to adult rats with the intention to prepare cortical membranes for [^{3}H]-ouabain binding assay. The results showed a significant increase in [^{3}H]-ouabain binding to membranes from L-NAME administered rats in the absence or presence of SST 10^{-6}M. In conclusion the SST produced changes in ATPase activity according to the moment in which the L-Name had been administered to experimental animals.

Keywords: **Nitric oxide, L-NAME; somatostatin; ATPase activity; high affinity [^{3}H]-ouabain.**

1. INTRODUCTION

Somatostatin (SST) is a cyclic peptide first isolated from ovine hypothalamus [1]. It is present in central and peripheral tissues whose main function is to inhibit the growth hormone secretion. This peptide can also inhibit hormone secretion and it has been implicated in antiproliferative signaling pathway modulation. Biological effects of somatostatin are due to its interaction to five receptor subtypes (somatostatin 1-5) that can be blocked by the antagonist cyclo-somatostatin (cSSTA) [2; 3]. Somatostatin behaves as a neuromodulador in CNS being involved in several psychiatric disorders such as schizophrenia. SST was reduced in the CFS of schizophrenics, levels that were recovered after haloperidol treatment [4; 5] and haloperidol-depot administration [6]. Besides, it has been reported that a reduction in SST mRNA expression in a subset of GABA neurons contributes to dorsolateral prefrontal cortex dysfunction in schizophrenia [7].

Nitric oxide (NO) is another molecule involved in schizophrenia [8; 9]. NO influence the maturation of neurons and synaptogenesis during the neuronal development. In consequence any disturbance in NO synthesis during the postnatal period interferes with the maturation of the brain neurons and their connections [10¸11]. For this reason, Nω-nitro-L-arginine methyl ester (L-NAME), a NOS inhibitor, produces biochemical alterations related to schizophrenia when it was administered during the postnatal period. This procedure has been considered as a model of schizophrenia, useful to

investigate drugs with a potential antipsychotic action [12; 13; 14]. However, L-NAME administration during the adulthood can produce a variety of effects, but not related to schizophrenia, for example, it produces an increase in the blood pressure and heart rate [15] a reduction in the hyperalgesia induced by modafinil [16] or a decrease in the locomotor activity after caffeine administration [17].

Na^+, K^+-ATPase is a pump that extrudes sodium out of the cells while potassium is pumping into the cells, against their concentration gradients and its activity is modulated by neuropeptides such as calcitonin, neurotensin among the others [18]. Therefore, the aim of this work was to study ATPase regulation by somatostatin in membranes isolated from adult rats which were administered with vehicle (saline) or L-NAME during the postnatal period or in the adulthood.

2. MATERIAL AND METHODS

Animals:

Male Sprague-Dawley rats were used in this study. 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. All animals were housed in a light-controlled and temperature controlled facility, with free access to food and water.

Reagents:

Somatostatin, Nω-nitro-L-arginine methyl ester, (cyclo-(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr [BZL]), ouabain, disodium ATP (grade I), were from Sigma Chemical Co., St. Louis, MO, USA. BCS Biodegradable counting scintillant liquid was from Amersham Biosciences, UK, and [^{3}H]-ouabain (Specific radioactivity 0.932 TBq / mmol) was from New England Nuclear, Du Pont, Boston Ma, USA.

Drug administration:

Six pups were injected with vehicle (saline solution) or 10 mg/kg L-NAME dissolved in saline solution on postnatal days 3, 4, and 5. The pups were returned to their mothers and no further manipulations were made until 56 postnatal days [12]. At that time, the animals were decapitated; cerebral cortices were harvested, homogenized and subjected to differential and sucrose gradient centrifugation to obtain synaptosomal membrane fractions [19]. For each L-NAME treated pooled pups, controls from saline injected pups were processed in parallel throughout. Furthermore, eight adult rats received a dose of 10 mg/kg L-NAME (i.p) that was dissolved in saline solution, thirty minutes before synaptosomal membrane preparation. For each L-NAME acute administration to adult rats, control rats were injected with saline solution and processed in parallel throughout.

Enzyme Assays:

ATPase activity was measured as described by Albers (1965) [20]. Synaptosomal membrane fractions were preincubated with 0.20 M Tris–HCl buffer (pH 7.4) or 10^{-9} M to 10^{-5} M somatostatin solution at 37ºC for 10 min. When indicated 10^{-9} M cSSTA was dissolved in bidistilled water and added during the preincubation period. 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 difference between activities was taken to correspond to Na^+, K^+-ATPase. This activity was monitored by colorimetric determination of orthophosphate released.

Isolation of Crude Membranes:

Cerebral cortices were pooled and in 0.32 M sucrose (neutralized to pH 7.0 with Tris base) and centrifuged at 900g for 10 min; supernatants were spun down at 100,000g for 30 min in a L90-Beckman ultracentrifuge. Prior to use, pellets were resuspended in redistilled water to achieve 10 mg protein per ml concentration and processed for [^{3}H]-ouabain binding assay.

Specific [^{3}H]-ouabain binding assay:

[^{3}H]-ouabain binding was carried out by a filtration assay [21]. Binding was performed in a medium (0.5 ml final volume) consisting of 3 mM $MgCl_2$, 2 mM H_3PO_4, 0.25 mM sucrose, 0.25 mM EDTA, 30 mM imidazol-HCl buffer (pH=7.4), 250 µg cerebral cortex membrane protein, 45 nM [^{3}H]-ouabain, and 10^{-6}M somatostatin dissolved in redistilled water to achieve the concentrations indicated. After incubation at 37ºC for 60 min, samples were filtered under vacuum on GF/B

filters positioned in a Millipore multifilter and rinsed twice with 2 ml of ice-cold 30 mM imidazol-HCl buffer, pH 7.4. Filters were transferred to vials and after addition of 10 ml counting scintillant liquid, the radioactivity was quantified in a Beckman Coulter-LS 6500 scintillation counter with 64% efficiency. Specific binding was calculated by subtracting binding found in the presence of 100 µM unlabeled ouabain. Non-specific binding accounted for less than 10% of total membrane-bound radioactivity.

Protein Measurement:

It was determined by the method of Lowry (1951) [22], using bovine serum albumin as standard.

Data Analysis:

Results were shown as mean values ± SD of n experiments Results were analyzed by "one sample Student 't' test" and by "one way ANOVA" followed by Dunnet's Multiple Comparison Test and Bonferroni Comparison Test. Probability level indicative of statistical significance was set at $P < 0.05$.

3. RESULTS AND DISCUSSION

Somatostatin (SST) effects on ATPase activity were recorded in synaptosomal membranes from rat cerebral cortex. Basal Na^+, K^+ -ATPase and Mg^{2+}-ATPase activities were 18.0 ± 5.3 µmol Pi x mg. $prot^{-1}$ x h^{-1} and 8,01 ± 3.95 µmol Pi x mg. $prot^{-1}$ x h^{-1}, respectively.

SST in a range of concentration of 10^{-9}-10^{-6} M not produce any change in these enzyme activities (Fig.1 A and B) But, SST at 10^{-5} M induced a simulator effect on Na^+, K^+ -ATPase activity. To determine whether somatostatin receptors were involved in the modulation of ATPase activity, further experiments were tested in the presence of cSSTA, a somatostatin receptor antagonist. The concomitant addition of cSSTA and somatostatin at nanomolar concentrations led to an inhibitory effect of Na^+, K^+ and Mg^{2+}-ATPase activities (Fig.2 A and B) suggesting that SST receptors could be involved in this inhibitory effect. Since when SST receptors were blocked, SST could bind to ATPase directly, inhibiting Na^+, K^+ -ATPase and Mg^{2+}-ATPase activities. SST behavior may resemble to the one recorded with neurotensin because it also inhibits Na^+, K^+ -ATPase through a competitive action directly on the enzyme. Several studies propose that NO may have a pathological role in schizophrenia, Black and colleagues [12] developed an animal model which consisted in postnatal administration of L-NAME, thus it is possible to interfere with NO production in early stages of the animal life producing behavioral and biochemical changes related to schizophrenia in the adulthood. It has been used this animal model to demonstrate changes in the rat behavior and neurotensin modulation on ATPase activity [23].

Na^+, K^+-ATPase activity was also assayed in synaptosomal membranes isolated from adult rats that were postnatal administered with vehicle (control) or 10 mg/kg L-NAME. These results demonstrated a significant reduction of the basal activity in L-NAME administered rats, which is consistent with results of clinical studies because it was reported that Na^+, K^+-ATPase activity was reduced in schizophrenic patients [24]. Somatostatin 10^{-6}M was added to the incubation media for ATPase assay and produced a significant stimulation of Na^+, K^+-ATPase activity in membranes from animals which were early administered with L-NAME (Fig. 3). Since ATPase located in nerve terminals is involved in neurotransmission it should be noted that SST reversed Na^+, K^+-ATPase inhibition allowing restore ionic balance altered by schizophrenia.

[^{3}H]-ouabain binding was assayed in crude membranes from adult rats which were injected with L-NAME or vehicle (control membranes) 30 min before membrane preparation. These results demonstrated that SST 10^{-6}M not produced any modification of [^{3}H]-ouabain binding when it was added to control membranes, but a significant increase was recorded in membranes from L-NAME injected rats (Fig. 4) in the absence or presence of the peptide. L-NAME acute administration would be producing a Na^+, K^+-ATPase conformational change which would facilitate ouabain binding to the enzyme.

4. CONCLUSION

SST effects on ATPase activity are influenced by animal age in which L-NAME was administered.

According to studies in schizophrenic patients, the model of schizophrenia implemented led to a reduction in Na^+, K^+-ATPase activity, an effect that was reversed by the presence of SST.

ACKNOWLEDGEMENTS

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APPENDIX - A

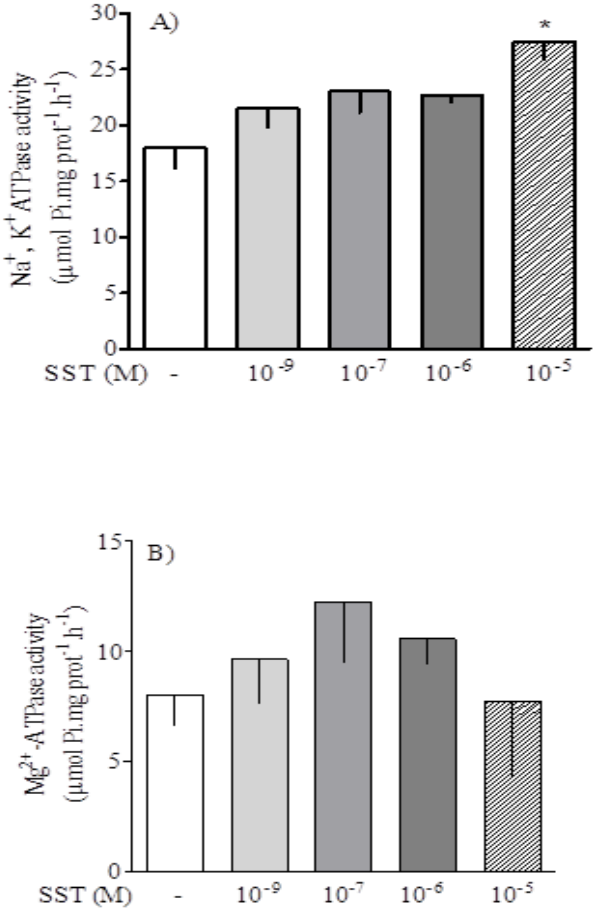

Fig.1: Somatostatin effect on A) Na^+, K^+-ATPase and B) Mg^{2+}-ATPase actvities. Cortical synaptosomal membranes were preincubated in the absence or presence of a range of concentration 10^{-9} M to 10^{-5} M somatostatin and assayed for ATPases. Results are expressed in µmoles de Pi x mg. prot $^{-1}$ x h $^{-1}$.

SD of 4-9 experiments performed per triplicate is indicated within the bars. *$P < 0.05$* respect to the control without somatostatin by one way ANOVA followed Dunnett's multiple comparison test.

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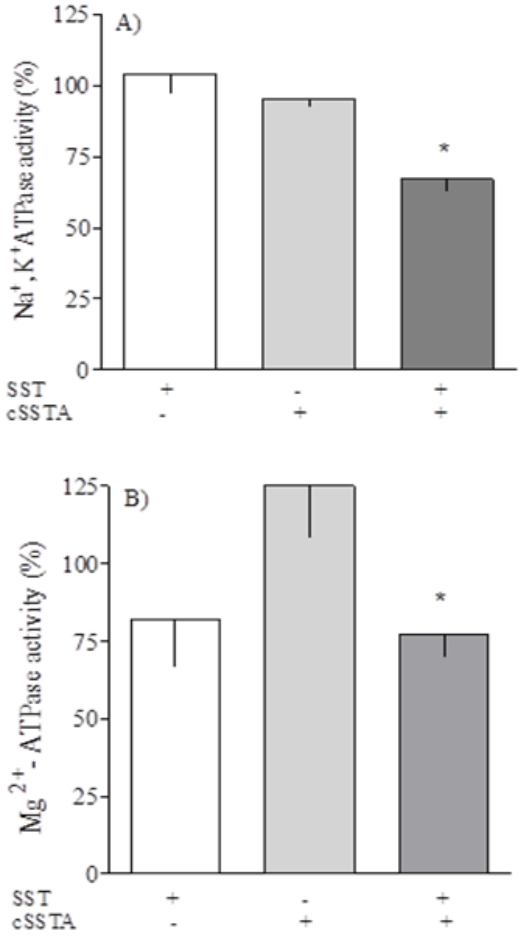

Fig.2: A) Na^+, K^+- ATPase activity and B) Mg^{2+}-ATPase activity in the presence of somatostatin and cSSTA, an antagonist of somatostatin receptors. Synaptosomal membranes from cerebral cortex of adult male rats were preincubated for 5 min with or without cSSTA, followed by 10^{-9} M somatostatin, for further 5 min period. Values for 4 experiments performed per triplicate are expressed as percentage enzyme activity (±SD), taking 100% data obtained in the absence of additions.

**P< 0.05* with respect to the control, by one sample Student t test.

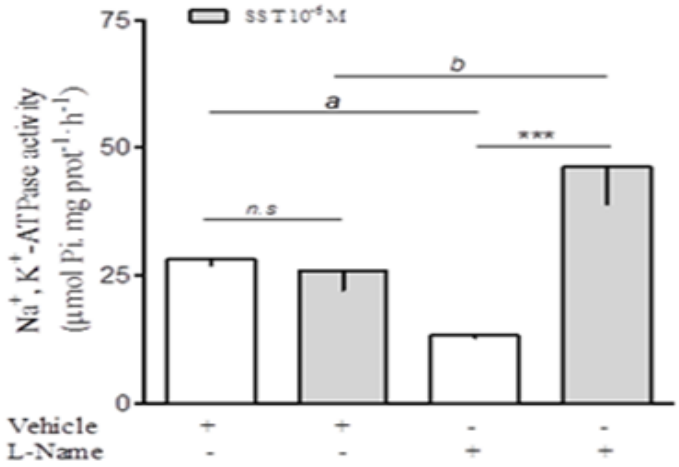

Fig.3: Somatostatin effect on Na^+, K^+- ATPase actvity in cerebral cortex of rats which were postnatally administered with vehicle (control) or 10 mg/kg L-NAME. Cortical synaptosomal membranes were preincubated in the absence or presence of 10^{-6} M somatostatin and assayed for ATPases. Results are expressed in µmoles de Pi x mg. prot $^{-1}$ x h $^{-1}$. SD of 4 experiments performed per triplicate is indicated within the bars. ***$P<0.001$ in somatostatin added to membranes from vehicle and L-NAME administered rats, respectively. $^{a}P< 0.05$ in L-NAME treated versus control; non-significance difference between saline solution and somatostatin added to vehicle treated rats was recorded.

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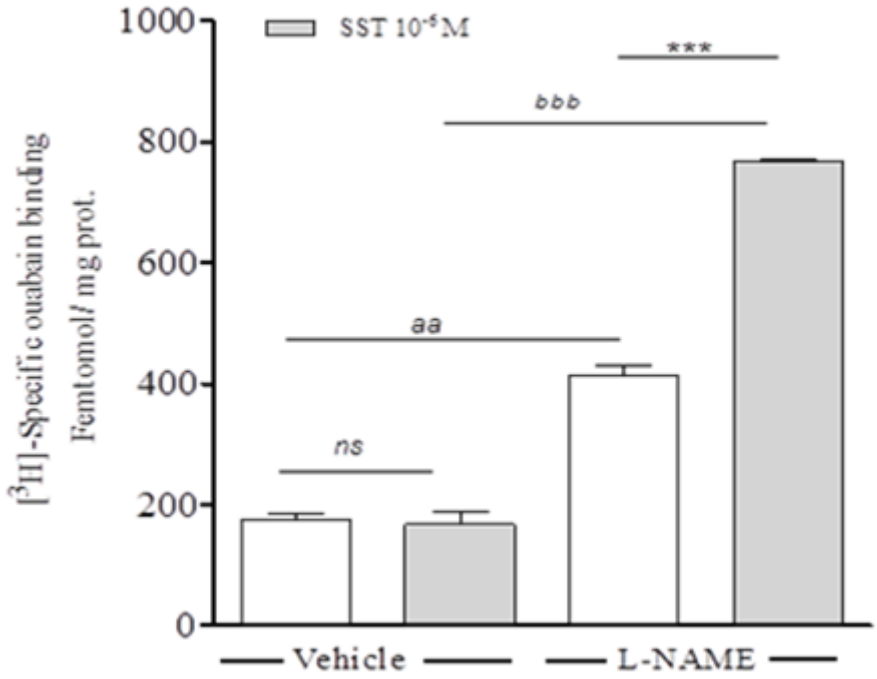

Fig. 4: [^{3}H]-ouabain binding was assayed after 10 mg/kg L-NAME i.p acute administration to adult rats. Bars represent mean values (±SD) from 4-5 experiments performed per triplicate.

Results are expressed in Femtomol x mg. prot^{-1}. Non-significance difference between vehicle (saline solution) and somatostatin added to control membranes; *** $P< 0.001$ between vehicle and somatostatin added to membranes from L-NAME administered rats; $^{aa}P<0.01$ in L-NAME treated versus control; $^{bbb}P<$ 0.001 somatostatin added to membranes from control and L-NAME treated rats by one way ANOVA followed Bonferroni Comparison Test.