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Neurotensin decreases high affinity [^{3}H]-ouabain binding to cerebral cortex membranes

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ABSTRACT

Previous work from this laboratory showed the ability of neurotensin to inhibit synaptosomal membrane Na^+, K^+-ATPase activity, the effect being blocked by SR 48692, a non-peptidic antagonist for high affinity neurotensin receptor (NTS1) [López Ordieres and Rodríguez de Lores Arnaiz 2000; 2001]. To further study neurotensin interaction with Na^+, K^+-ATPase, peptide effect on high affinity [^{3}H]-ouabain binding was studied in cerebral cortex membranes. It was observed that neurotensin modified binding in a dose-dependent manner, leading to 80% decrease with 1×10^{-4} M concentration. On the other hand, the single addition of 1×10^{-6} M, 1×10^{-5} M and 1×10^{-4} M SR 48692 (Sanofi-Aventis, U.S., Inc.) decreased [^{3}H]-ouabain binding (in %) to 87 ± 16; 74 ± 16 and 34 ± 17, respectively. Simultaneous addition of neurotensin and SR 48692 led to additive or synergic effects. Partial NTS2 agonist levocabastine inhibited [^{3}H]-ouabain binding likewise. Saturation assays followed by Scatchard analyses showed that neurotensin increased K_d value whereas failed to modify B_{max} value, indicating a competitive type interaction of the peptide at Na^+, K^+-ATPase ouabain site. At variance, SR 48692 decreased B_{max} value whereas it did not modify K_d value. [^{3}H]-ouabain binding was also studied in cerebral cortex membranes obtained from rats injected i. p. 30 min earlier with 100 μg and 250 μg/kg SR 48692. It was observed that the 250 μg/kg SR 48692 dose led to 19% decrease in basal [^{3}H]-ouabain binding. After SR 48692 treatments, addition of 1×10^{-6} M led to additive or synergic effect. Results suggested that [^{3}H]-ouabain binding inhibition by neurotensin hardly involves NTS1 receptor.

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1. Introduction

Neurotensin is a peptide widely distributed throughout the gastrointestinal tract and the central nervous system [1]. Neurotensinergic system interacts with other neurotransmitter systems, including dopaminergic, cholinergic, serotonergic, opioid and aminoacidergic systems, among others [2].

Neurotensin binds to a group of receptors [3,4]. Two of them, termed NTS1 and NTS2, are seven transmembrane domain receptors coupled to G proteins, which bind neurotensin with high and low affinity, respectively [4]. This peptide acts as an agonist for all NTS1-mediated pathways, whereas it may exert either agonist or antagonist activities according to the NTS2 mediated pathway involved [5]. Another two neurotensin receptor types are mainly intracelullar receptors and are termed NTS3/sortilin and nts4/SorLA [6].

During the transmission of the nervous impulse, sodium and potassium ions move through neuronal membranes. Maintenance and restoration of ionic equilibria take place with the participation of the sodium pump; the enzymatic version of the sodium pump is the Na^+, K^+-ATPase. Modulation of this mechanism is essential not only at rest but also after the passage of the nervous impulse.

Na^+, K^+-ATPase is a transmembrane enzyme with an intracellular site for Mg^{2+}-ATP and Na^+ and an extracellular site for K^+, which binds ouabain and other cardiotonic glycosides. Ouabain site (K^+ site) is regulatory for the enzyme activity. This enzyme catalyzes the hydrolysis of ATP in a two steps process: a phosphorylation step and a dephosphorylation step, K^+-dependent, sensitive to ouabain. In the later step, it behaves as a non-specific phosphatase (*p*-NPPase), hydrolyzing other phosphates, including *p*-nitrophenylphosphate (*p*-NPP), a non-natural substrate [7].

Neurotensin inhibits the activity of Na^+, K^+-ATPase in synaptosomal membranes, known to be enriched in this enzyme activity [8]. Binding studies disclosed that central nervous system membranes bind ouabain with high, intermediate and low affinity, corresponding to α3, α2 and α1 isoforms of Na^+, K^+-ATPase catalytic subunit. The α3 isoform predominates in neurons [9–11].

Experimental evidence suggests that neurotensin may modulate ionic transport at neuronal membranes, due to its ability to modify in vitro the activity of Na^+, K^+-ATPase. Previous work performed in cerebral cortex synaptosomal membranes disclosed that Na^+, K^+-ATPase activity is inhibited by neurotensin [12] and that K^+-*p*-nitrophenylphosphatase

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(K^+-*p*-NPPase) activity is likewise inhibited [13] but only under strict experimental conditions. The antagonist for high affinity neurotensin (NTS1) receptor named SR 48692 [14], invariably blocked the neurotensin mentioned effect [12,13].

In order to further explore whether the K^+ site was involved in neurotensin effect on Na^+, K^+-ATPase activity, herein the effect of neurotensin on high affinity [^{3}H]-ouabain binding to cerebral cortex membranes was tested. Assays were also carried out in the presence of levocabastine or SR 48692, as well as after the administration of the later. In all cases, [^{3}H]-ouabain binding was diminished. Results suggested that [^{3}H]-ouabain binding inhibition by neurotensin hardly involves NTS1 receptor.

2. Materials and methods

2.1. Animals and drugs

Adult male Wistar rats weighing 100–150 g were used. 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. Reagents were analytical grade. Ouabain and neurotensin acetate were from Sigma Chemical Co., St. Louis, MO, U.S.A.; peptide solutions in bidistilled water were freshly prepared for each experiment. Dimethylsulfoxide (DMSO) was from J. T. Baker Chemical Co., Phillipsburg, N. J., USA. BCS Biodegradable counting scintillant was from Amersham Biosciences, U.K., and [^{3}H]ouabain (specific radioactivity of 20.5 Ci / mmol) was from New England Nuclear, Du Pont, Boston, MA, U.S.A. SR 48692 {2-[(1-(7-chloro-4-quinolinyl)-5-(2,6-dimethoxy phenyl) pyrazol 3-yl) carbonylamino]tricyclo (3.3.1.1 $^{3.7}$) decan-2-carboxylic acid}and levocabastine clorhidrate were kindly provided by Sanofi-Aventis, U.S., Inc., and Jansen-Cilag, Argentina, respectively.

2.2. Drug administration

SR 48692 administration was carried out according to Brun et al. [15]. Six rats were used in each experiment. Groups of two rats received i.p. 100 or 250 μg/kg SR 48692 suspended in saline solution with 0.01% Tween 80 (vehicle) and the other two received vehicle. Thirty minutes later, animals were killed by decapitation, skulls opened and cerebral cortices harvested. This protocol was repeated three times.

2.3. Isolation of crude cerebral cortex membranes

Cerebral cortices of 3 rats were pooled and homogenized for two 1-min periods in 0.32 M sucrose (neutralized to pH 7 with Tris base) and centrifuged at 900 *g* for 10 min; supernatants were spun down at 100,000 *g* for 30 min in a L8-Beckman ultracentrifuge. Resultant pellets were resuspended in 0.16 M sucrose and distributed in 8 tubes for further spinning as above, and after discarding the supernatant, stored at −70 °C. Prior to use, pellets were resuspended in bidistilled water to achieve a concentration of 5 mg protein per ml, and processed for [^{3}H]-ouabain binding assay.

2.4. [^{3}H]-Ouabain binding

[^{3}H]-ouabain binding was carried out by a filtration assay using [^{3}H]-ouabain. Binding was performed in triplicate 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 [16], 250 μg cerebral cortex membranes protein and 45 nM [^{3}H]-ouabain, unless otherwise stated. When indicated, neurotensin, levocabastine and /or SR 48692 were included in the incubation medium. DMSO 10% was added to the controls in order to isolate the effect of SR 48692 from the solvent.

After incubation at 37 °C 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 9 ml of counting scintillant, radioactivity was quantified in a Beckman Coulter-LS 6500 scintillation counter with 65% efficiency. Specific binding was calculated by substracting binding found in the presence of 100 μM unlabelled ouabain. Non-specific binding accounted for less than 10% of total membrane-bound radioactivity.

To obtain a competition curve for neurotensin on [^{3}H]-ouabain binding assays were carried out in the presence of several neurotensin concentrations.

For saturation binding assays, triplicate membrane samples were incubated in the presence of ligand ouabain at the concentrations indicated. For ouabain concentrations higher that 45 nM, unlabelled ouabain was added to achieve the required ligand concentrations. The neurotensin curve contained 2×10^{-5} M peptide dissolved in bidistilled water. This peptide concentration was chosen because it was near the Ki value recorded. The control curve was performed in the absence of neurotensin. The SR 48692 curve contained this drug at 2×10^{-5} M concentration, dissolved in DMSO 10% (V/V). The control curve in the absence of SR 48692 contained DMSO 10% (V/V).

2.5. Protein measurement

Protein was determined by the method of Lowry et al. [17] using bovine serum albumin as standard.

2.6. Data analysis

Data are presented as mean values ± SD of *n* experiments. Values were compared by one-sample Student's *t*-test or by Student's *t*-test as indicated.

The probability level indicative of statistical significance was set at $P<0.05$.

For saturation assays, non-linear regression of the data were processed using GraphPad Prism program (version 4.0); the best fit to the data was determined according to *F*-tests. Constant values were calculated by non-linear regression fits. Scatchard transformation of saturation binding data showed that only one receptor population was operative.

3. Results

High affinity [^{3}H]-ouabain binding to cerebral cortex membranes was determined in several experimental conditions. Basal values ranged from 506 to 935 fmol ($n=6$) per mg protein. A competition curve for [^{3}H]-ouabain binding *versus* neurotensin concentration was determined. Results recorded were averaged and a Ki value of 2.12×10^{-5} M was obtained (Fig. 1).

Binding was inhibited in a dose-dependent manner by neurotensin and SR 48692. Results showed that neurotensin and SR 48692 led to similar decreases on [^{3}H]-ouabain binding according to the concentration tested. In the presence of either substance at 1×10^{-4} M concentration, inhibition reached respectively 78% and 66% (Fig. 2A and B).

High affinity [^{3}H]-ouabain binding was studied in the presence of the simultaneous addition of neurotensin plus SR 48692. In the single presence of neurotensin or SR 48692 both at 1×10^{-6} M concentration, binding was reduced to 92% and to 85%, respectively. In the presence of the joint addition of both drugs at 1×10^{-6} M concentration, [^{3}H]-ouabain binding decreased to 60%, indicating that the effect is more than additive. With neurotensin or SR 48692, both at 1×10^{-5} M concentration, binding decreased roughly to 75%. With neurotensin plus the antagonist, both at 1×10^{-5} M concentration, binding decreased to 42% (Fig. 3).

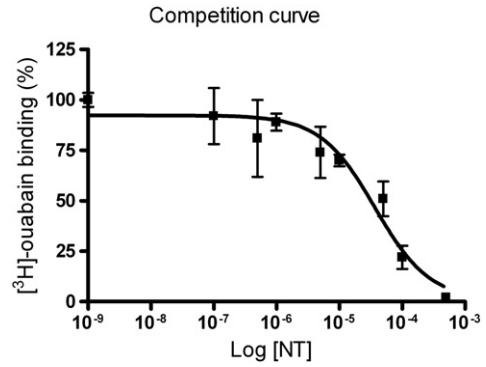

Fig. 1. Competition curve for [^{3}H]-ouabain binding versus neurotensin. Cerebral cortex membranes were incubated in the absence or presence of neurotensin at the concentrations indicated. Results were obtained in 3–8 assays carried out with 4 different membranes preparations. Data are expressed as percent of control values obtained in the absence of neurotensin.

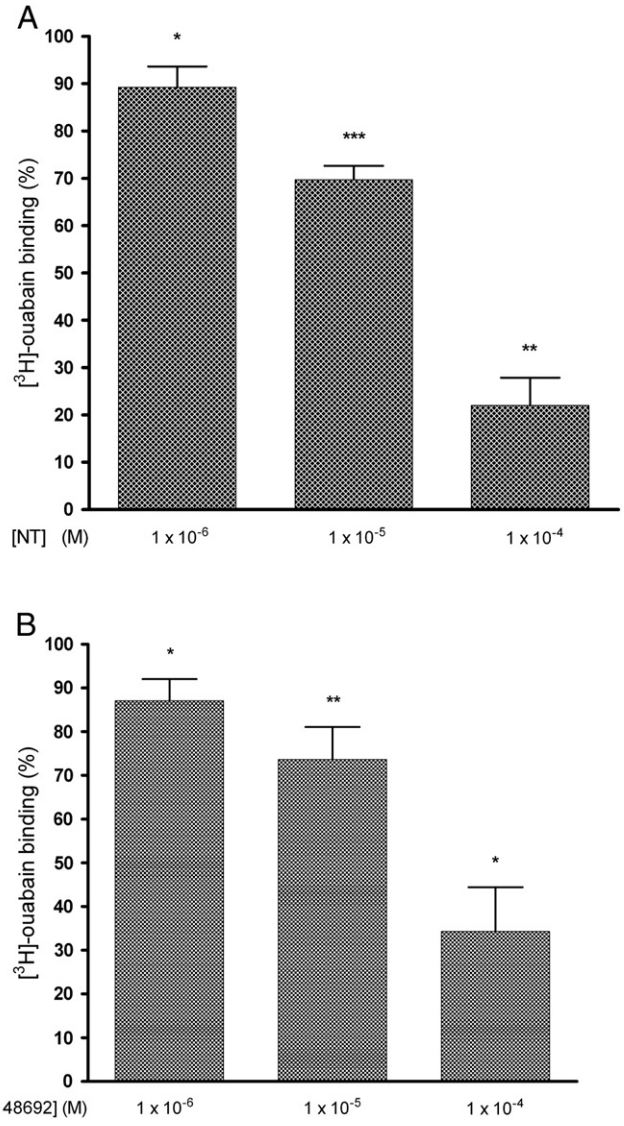

Fig. 2. Effect of agonist neurotensin and antagonist SR 48692 on high affinity [^{3}H]-ouabain binding. Cerebral cortex membranes were incubated in the absence or presence of neurotensin (A) and SR 48692 (B) at the concentrations indicated. Results are expressed as per cent binding taken as 100% values recorded in the absence of additions. Data are mean values (±SD) from 3 to 11 experiments. NT, neurotensin. *P<0.05; **P<0.01; ***P<0.001, by Student's one sample t-test.

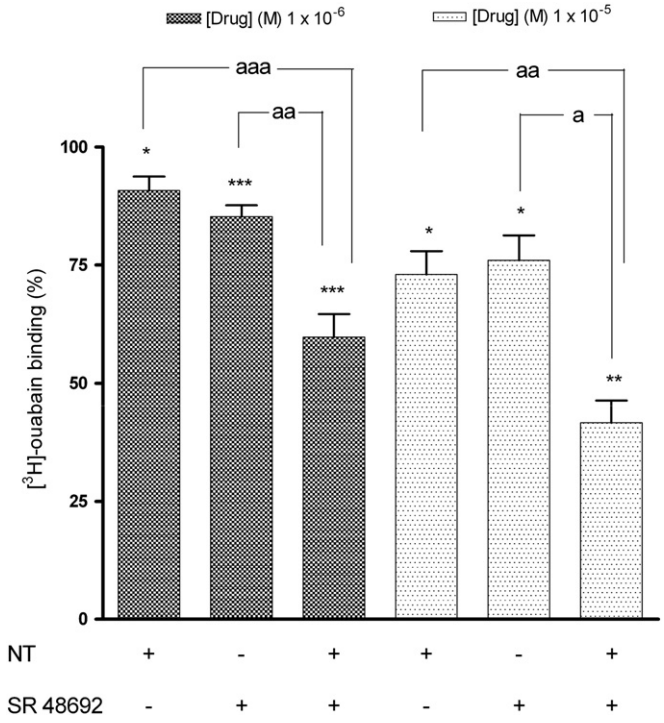

Fig. 3. Effect of the simultaneous presence of neurotensin and SR 48692 on high affinity [^{3}H]-ouabain binding. Cerebral cortex membranes were incubated in the absence or presence of neurotensin and/or SR 48692 at the concentrations indicated. Results are expressed as % binding taken as 100% values recorded in the absence of additions. Data are mean values (±SD) from 3 to 7 experiments. NT, neurotensin. *P<0.05; **P<0.01; ***P<0.001, by Student's one sample t-test. $^a P$<0.05; $^{aa} P$<0.01; $^{aaa} P$<0.001, by Student's t-test.

Saturation curves of [^{3}H]-ouabain binding to cerebral cortex membranes were carried out in the absence and presence of neurotensin at a range of 2.8–360 nM concentration. Five experiments were performed in different occasions. Scatchard analysis showed that in every experiment K_d value invariably increased whereas B_{max} value remained unchanged. K_d values in nM (means ± SD), in the absence and presence of neurotensin, were respectively 60.53 ± 3.56 and 148.0 ± 7.1, indicating a decrease in receptor affinity for the ligand. B_{max} values were 1622 ± 31 and 1484 ± 29 fmol.mg prot.$^{-1}$ in the absence and presence of neurotensin, respectively (means ± SD) (Fig. 4A).

Saturation curves of [^{3}H]-ouabain binding to cerebral cortex membranes were carried out in the absence and presence of SR 48692 dissolved in DMSO 10% (V/V) at a range of 5–360 nM concentration. Three experiments were performed in different occasions. Scatchard analysis showed that in every experiment B_{max} value invariably decreased whereas K_d value remained unchanged. B_{max} values were respectively 6820 ± 324 and 4737 ± 119 fmol. mg prot. $^{-1}$ in the absence and presence of SR 48692 (means ± SD). K_d values in nM, in the absence and presence of SR 48692, were respectively 109.1 ± 12.5 and 101.4 ± 6.5 (means ± SD) (Fig. 4B).

In order to explore potential involvement of NTS2 receptor in [^{3}H]-ouabain binding inhibition here described, experiments with NTS2 receptor ligand levocabastine were carried out. With 1×10^{-6} M levocabastine only a trend to decrease [^{3}H]-ouabain binding was recorded, whereas with 1×10^{-5} M levocabastine, a statistically significant decrease (28%) on this ligand binding was obtained (Fig. 5).

High affinity [^{3}H]-ouabain binding to cerebral cortex membranes was determined after i.p. administration of SR 48692 (dissolved in saline solution with 0.01% Tween 80). A tendency to decrease high affinity [^{3}H]-ouabain binding was recorded after the dose of 100 μg/kg SR 48692 whereas a statistically significant decrease in binding (19%) was obtained after the dose of 250 μg/kg SR 48692 (Fig. 6). In

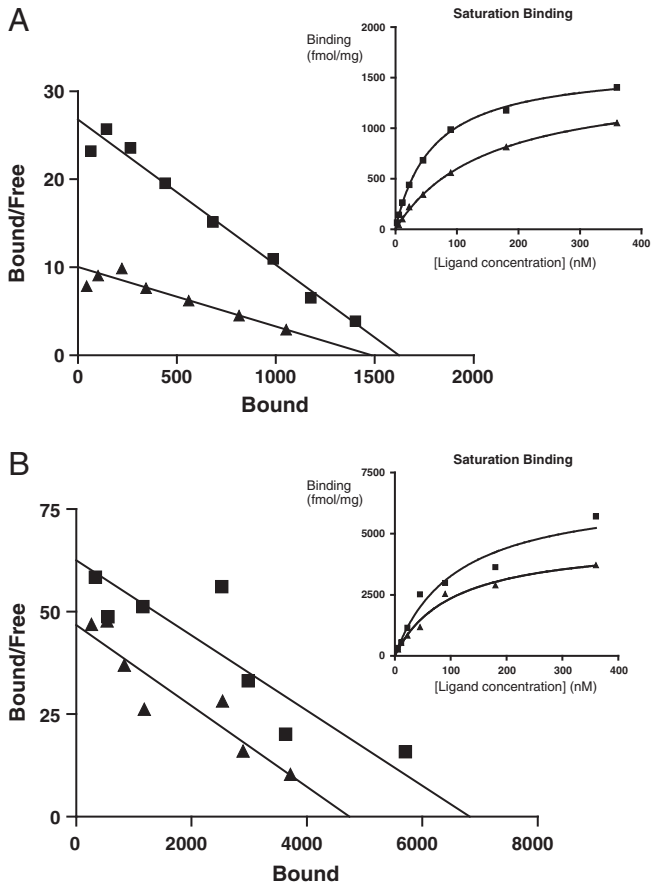

Fig. 4. Scatchard plots for [^{3}H]-ouabain binding to cerebral cortex membranes at equilibrium in the absence (■) or presence (▲) of 2×10^{-5} M neurotensin (A) or 2×10^{-5} M SR 48692 dissolved in DMSO 10% (V/V) (B). Each point in the figures represents the mean value from five or six (A) or three (B) membrane samples, each assayed in triplicate. Inset, saturation curves.

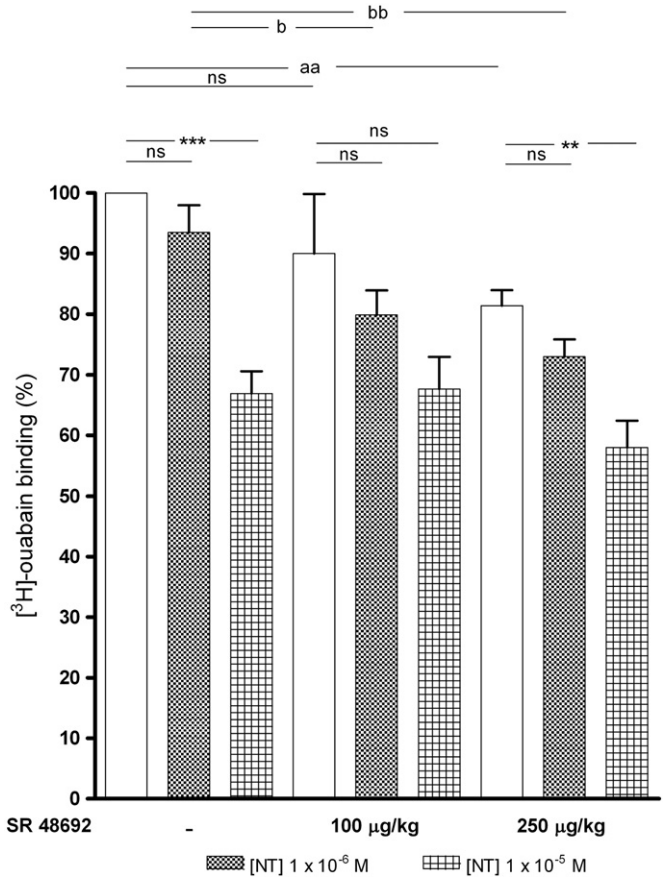

Fig. 6. Effect of neurotensin on high affinity [^{3}H]-ouabain binding after administration of SR 48692. Cerebral cortex membranes were obtained 30 min after i. p. administration of SR 48692 or vehicle and processed for high affinity [^{3}H]-ouabain binding in the absence or presence of neurotensin at the concentations indicated. Results are expressed as per cent binding taken as 100% the basal value recorded after administration of the vehicle in the absence of additions. Data are mean values (±SD) from 3 to 11 experiments. NT, neurotensin. $^{**}P<0.01$; $^{***}P<0.001$ versus the corresponding control without neurotensin, by Student's one sample *t*-test. $^{aa}P<0.01$ versus vehicle injected, by Student's one sample *t*-test. $^{b}P<0.05$; $^{bb}P<0.01$ versus vehicle injected, all with 1×10^{-6} M neurotensin, by Student's *t*-test. ns, non-significant difference.

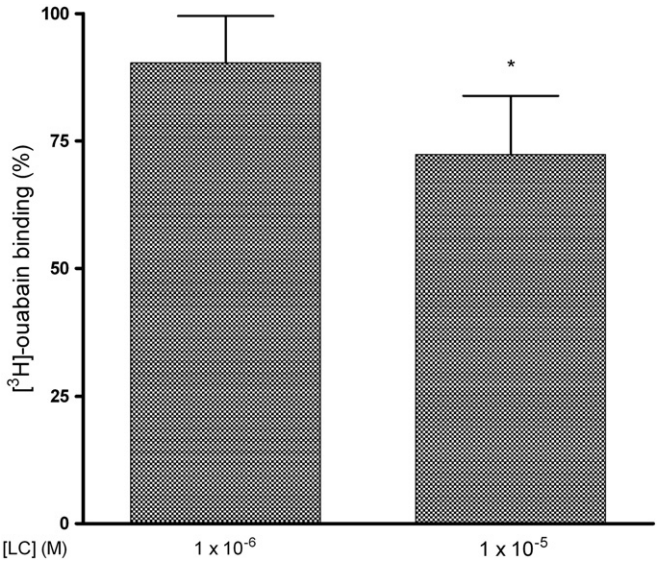

Fig. 5. Effect of partial NTS2 agonist levocabastine on high affinity [^{3}H]-ouabain binding. Cerebral cortex membranes were incubated in the absence or presence of levocabastine at the concentrations indicated. Results are expressed as per cent binding taken as 100% values recorded in the absence of additions. Data are mean values (±SD) from 6 to 8 experiments. LC, levocabastine. $^{*}P<0.05$, by Student's one sample *t*-test.

membranes obtained from control rats injected with the vehicle, only a trend to decrease binding was recorded with 1×10^{-6} M neurotensin whereas roughly a 30% decrease was obtained with 1×10^{-5} M peptide. After administration of 100 µg/kg SR 48692, neurotensin addition only led to a tendency to decrease binding. After a 250 µg/kg SR 48692 dose, a statistically significant decrease was only recorded with neurotensin at 1×10^{-5} M concentration. It may be mentioned that differences achieved statistically significance when data obtained with 1×10^{-6} M neurotensin were compared *inter se*, that is, vehicle injected *versus* the two SR 48692 doses (Fig. 6, columns 2, 4 and 6). Experiments carried out with 1 mg/kg SR 48692 failed to show differences *versus* those recorded with the dose of 250 µg/kg SR 48692 (data not shown).

4. Discussion

Previous work showed that neurotensin inhibits Na^+, K^+-ATPase activity, an effect which is prevented by NTS1 antagonist SR 48692 (see Introduction). To analyze further potential effect of neurotensin on Na^+, K^+-ATPase K^+ site, high affinity [^{3}H]-ouabain binding to cerebral cortex membranes was assayed. Results indicated that the peptide dose-dependently decreased ouabain binding and that NTS1 antagonist SR 48692 not only failed to prevent such effect but exerted itself an inhibitory action on ligand binding. The joint addition of neurotensin plus SR 48692 lead to additive or synergic effect. Scatchard

analyses indicated differences between both inhibitors. Partial NTS2 agonist levocabastine inhibited [^{3}H]-ouabain binding likewise. [^{3}H]-ouabain binding decreased after intraperitoneal administration of SR 48692, an effect which was enhanced by further addition of neurotensin *in vitro*.

Neurotensin effect here described on high affinity [^{3}H]-ouabain binding may be due to a direct effect, similar to other actions described for peptides on ligand binding. To illustrate, both neurotensin [18] and calcitonin [19] diminish the binding of ligand [^{3}H]-quinuclidinyl benzilate (QNB) to muscarinic cholinergic receptor. Present results suggested an interaction of the peptide with Na^+, K^+-ATPase at the enzyme K^+ site. Alternatively, an effect mediated by neurotensin receptors may be operative.

At CNS neurotensin is involved in several physiological processes which include locomotion, modulation of stress and pain, among others [20]. Besides, the peptide can produce profound analgesia [21–23]. Neurotensin behaves similar to ouabain in some experimental models. Intracerebroventricular administration of this cardenolide produces a dose-dependent decrease in locomotor activity which is correlated with ERK1/2 phosphorylation [24]. When centrally administered, neurotensin likewise decreases locomotion [25] and catalyses ERK phosphorylation [26].

In most experimental models, SR 48692 behaves as a potent antagonist for high affinity neurotensin receptors class. Hypolocomotion induced by neurotensin is antagonized by SR 48692 [27] and it cannot be observed in deficient NTS1 receptor mice [28], indicating that NTS1 receptor is involved in this effect. However, in other studies SR 48692 failed to antagonize neurotensin induced hypolocomotion [29]. This drug fails to antagonize electrophysiological neurotensin effect on evoked current mediated by high affinity neurotensin receptor in a *Xenopus oocytes* expression system [30] and high affinity [^{3}H]-ouabain binding decrease by neurotensin (present findings).

Both agonist neurotensin and antagonist SR 48692 for NTS1 receptor decreased high affinity [^{3}H]-ouabain binding. The extent of the effect recorded was almost identical at equimolecular concentration of these drugs. From a pharmacological point of view it is difficult to explain that both substances behave similarly. Herein experiments were not carried out in a whole system. Therefore, a pharmacological response mediated by a receptor followed by activation of a intracellular cascade does not occur.

The joint addition of neurotensin plus SR 48692 lead to additive or synergic effect. This finding suggested that at least two different mechanisms seemed to be involved in this effect. Saturation binding parameters for high affinity [^{3}H]-ouabain binding clearly differed between neurotensin *versus* SR 48692. Neurotensin failed to modify Bmax whereas it increased Kd value, indicating a decrease in binding affinity. This finding may be related to the increased Kd value for high affinity [^{3}H]-ouabain binding to CNS membranes reported after sleep deprivation [31]. In support, a decrease in Kd value after neuronal hyperactivity induced by repeated electroconvulsive shock was observed [32]. On the other hand, antagonist SR 48692 modified Bmax but failed to alter Kd value (present results). These findings suggested that neurotensin and SR 48692 act through distinct mechanisms.

The comparison of control ouabain saturation curves (without additions) in neurotensin *versus* SR 48692 experiments showed that Kd values were rather similar whereas Bmax values markedly differed. Bmax values were higher in SR 48692 experiments *versus* those recorded in neurotensin experiments, most likely due to the presence of DMSO which was employed to dissolve SR 48692.

Present findings indicated that neurotensin, SR 48692 and levocabastine inhibited [^{3}H]-ouabain binding. It is known that these three substances behave as agonists or antagonists at NTS-2 receptor, depending on the specific signaling events and cell types under examination [33]. It may be postulated that both levocabastine and SR 48692 inhibited [^{3}H]-ouabain binding by a mechanism involving agonist effect on NTS2 receptor. These assays were performed in membrane fractions where cell structures, Na^+/K^+-ionic gradients and cell to cell responses cannot occur. If this study had been carried out in a whole system, potential signalling mechanisms could have been taken into account. It is worthwhile to mention that several peptide antagonists bind to the same site or that they overlap sites on NTS3 receptor [34,35].

It is reasonable to think that NTS2 receptor and Na^+, K^+-ATPase are closely related in the synaptic membranes and that some interaction between them may be operative. This may led to a conformational enzyme change, with a decrease in ligand ouabain to K^+ enzyme site.

SR 48692 administration failed to prevent further neurotensin effect on [^{3}H]-ouabain binding but itself decreased [^{3}H]-ouabain binding. The effect seemed to be permanent, because it resisted tissue homogenization and differential centrifugation steps, which are required for the preparation of the membranes previous to ligand binding assays. This fact gives a potential functional consequence for the *in vitro* effect shown herein. When added together at low concentrations, neurotensin and SR 48692 led to an inhibitory effect on ouabain binding which was more than additive. After SR 48692 administration a statisticaly significant decrease was observed by further addition of 1×10^{-6} M neurotensin, a concentration which failed to inhibit binding in membranes isolated from vehicle treated rats. Taken jointly, these findings indicated a synergic effect, suggesting again that at least two different mechanisms may be involved in this action.

In a previous study the potential participation of NTS1 receptor in phosphoinositide hydrolysis enhancement by inhibition of the sodium pump was evaluated. Results showed that antagonist SR 48692 blocks the inhibitory effects of ouabain on sodium pump modulating phosphoinositide hydrolysis. This result suggests that high-affinity neurotensin receptors are involved, at least partially, in the process [36]. These findings may receive a new interpretation in the ligth of present results. Herein we showed an interference between SR 48692 and ouabain at the Na^+, K^+-ATPase ouabain binding site. Therefore, it seems reasonable that the phosphoinositide hydrolysis increase by Na^+, K^+-ATPase inhibitors is prevented by *in vitro* addition of SR 48692 [36].

Neurotensin, SR 48692 and levocabastine separately decreased [^{3}H]-ouabain binding to Na^+, K^+-ATPase. On the other hand, neurotensin is able to diminish *in vitro* Na^+, K^+-ATPase activity present in purified synaptosomal membranes [12,13]. It is known that ouabain binds to K^+-site of Na^+, K^+-ATPase [see 7]. Because neurotensin effect on [^{3}H]-ouabain binding proved to be competitive type, a steric impairement at the enzyme K^+ site may take place.

Preliminary results for [^{3}H]-ouabain binding to cerebral cortex membranes after neurotensin i.c.v. administration showed a statistically significant decrease in ligand binding (data not shown). This indicates that neurotensin *in vitro* effect may well be of functional significance because it may be reproduced by peptide administration.

A previous study showed that SR 48692 itself fails to alter ATP hydrolysis whereas it prevents Na^+, K^+-ATPase inhibition by neurotensin [12]. Present results indicated that SR 48692 exerted *per se* an inhibitory effect on high affinity [^{3}H]-ouabain binding to cerebral cortex membranes and that this antagonist failed to prevent neurotensin inhibitory action on high affinity [^{3}H]-ouabain binding. Therefore, it is concluded that the effect of SR 48692 is entirely different when hydrolysis of ATP or high affinity [^{3}H]-ouabain binding of ATPase are evaluated.

Results summarized indicated that neurotensin decreased [^{3}H]-ouabain binding to cerebral cortex membranes. Findings showed that neurotensin produced competitive type inhibition of [^{3}H]-ouabain binding, with increased Kd value. It is postulated that neurotensin interacts with Na^+, K^+-ATPase K^+ site, either directly or through a neurotensin receptor. This effect was not blocked by *in vitro* addition of SR 48692 or by previous i.p. administration of this antagonist. Such findings led us to conclude that this neurotensin

effect hardly involves NTS1 receptor. Besides, the single presence of SR 48692 or levocabastine also diminished [^{3}H]-ouabain binding and the simultaneous addition of neurotensin and SR 48692 resulted in additive or synergic effects. Because SR 48692 and levocabastine behave as agonists for NTS2 receptor in some experimental models [30,33], the suggestion that this receptor may be involved in neurotensin effect on [^{3}H]-ouabain binding is advanced.

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