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ORIGINAL PAPER

High-affinity Neurotensin Receptor is Involved in Phosphoinositide Turnover Increase by Inhibition of Sodium Pump in Neonatal Rat Brain

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Abstract Phosphoinositide (PI) metabolism is enhanced in neonatal brain by activation of neurotransmitter receptors and by inhibition of the sodium pump with ouabain or endogenous inhibitor termed endobain E. Peptide neurotensin inhibits synaptosomal membrane Na^+, K^+-ATPase activity, an effect blocked by SR 48692, a selective antagonist for high-affinity neurotensin receptor (NTS1). The purpose of this study was to evaluate potential participation of NTS1 receptor on PI hydrolysis enhancement by sodium pump inhibition. Cerebral cortex miniprisms from neonatal Wistar rats were preloaded with [^{3}H]*myo*inositol in buffer during 60 min and further preincubated for 0 min or 30 min in the absence or presence of SR 48692. Then, ouabain or endobain E were added and incubation proceeded during 20 or 60 min. Reaction was stopped with chloroform/methanol and [^{3}H]inositol-phosphates (IPs) accumulation was quantified in the water phase. After 60-min incubation with ouabain, IPs accumulation values reached roughly 500% or 860% in comparison with basal values (100%), if the preincubation was omitted or lasted 30 min, respectively. Values were reduced 50% in the presence of SR 48692. In 20-min incubation experiments, IPs accumulation by ouabain versus basal was 300% or 410% if preincubation was 0 min or 30 min, respectively, an effect blocked 23% or 32% with SR 48692. PI hydrolysis enhancement by endobain E was similarly blocked by SR 48692, being this effect higher when sample incubation with the endogenous inhibitor lasted 60 min versus 20 min. Present results indicate that PI hydrolysis increase by sodium pump inhibition with ouabain or endobain E is partially diminished by SR 48692. It is therefore suggested that NTS1 receptor may be involved in cell signaling system mediated by PI turnover.

Keywords Neurotensin receptors · Phosphoinositide hydrolysis · Na^+, K^+-ATPase inhibitors · Neonatal brain cortex · Cell signaling

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Introduction

Activation of several brain neurotransmitter receptors leads to phosphoinositide (PI) turnover enhancement [1, 2]. Muscarinic agonists as carbachol, a nonhydrolyzable analog of acetylcholine, stimulate the accumulation of inositol phosphate (IP) in rat brain cortex, an effect which peaks at postnatal day seven [3]. In addition, the high-affinity neurotensin (NTS1) receptor, a G-protein receptor involved in IP accumulation [4], is present in brain at birth and peaks at day seven [5]. Experimental evidence points to a relationship between cholinergic and neurotensinergic systems; accordingly, PI hydrolysis induced by carbachol, either alone or plus neurotensin is partially or totally blocked by SR 48692, a non-peptidic antagonist for neurotensin

(NTS1) receptor [6], suggesting the involvement of this receptor in carbachol effect [7].

The metabolism of PI is also enhanced with ouabain, the specific and selective inhibitor of the sodium pump. It is known that the sodium pump (Na^+, K^+-ATPase, EC 3.6.1.3), is essential for the maintenance and restoration of Na^+/K^+ equilibrium through neuronal membranes. Therefore, its regulation could help understand molecular mechanisms involved in neurotransmission [8, 9]. In the search for endogenous Na^+, K^+-ATPase modulators, diverse efforts have been devoted and results obtained have been summarized [10–13]. In our laboratory, a soluble brain fraction (peak II) was isolated [14], which besides inhibiting Na^+, K^+-ATPase activity also shares several properties with ouabain [11], thus suggesting the term *endobain*. Fractionation of peak II by ionic exchange HPLC led to a more purified Na^+, K^+-ATPase inhibitory fraction, II-E [15], which is structurally different from the plant-origin cardiac glycoside [16] but is also able to block high-affinity [^{3}H]ouabain binding [17] and to induce neurotransmitter release [18], justifying its denomination as *endobain E* [12].

Similar to PI hydrolysis enhancement by activation of diverse neurotransmitter receptors, IP accumulation increase by ouabain is markedly higher in neonatal in comparison to adult brain [19]. Endogenous Na^+, K^+-ATPase inhibitor endobain E also enhances PI turnover in neonatal brain but not in adult tissue [20].

The purpose of the present study was to evaluate potential participation of NTS1 receptor in PI hydrolysis enhancement by inhibition of the sodium pump. Experiments performed in cerebral cortex prisms from neonatal rats in the presence of ouabain or endobain E with SR 48692 indicated that high-affinity neurotensin receptors are involved, at least partially, in the process.

Experimental Procedure

Materials

Reagents were analytical grade. Ouabain and *myo*inositol were purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) was purchased from J.T. Baker Chemical Co. (Phillipburg, NJ). A Dowex anion exchange resin (AG 1-X8, 100–200 mesh, formate form) was from Bio-Rad Laboratories (Richmond, CA, USA). 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} was kindly provided by SANOFI Recherche, France. OptiPhase "Hisafe" 3 was purchased from Wallac Oy (Turku, Finland). [^{3}H]*myo*inositol (20 Ci/mmol) was from New England Nuclear (Boston, MA, USA).

Animals

For the preparation of brain prisms, Wistar neonatal rats (6–7 days old) of either sex were used, considering "day 0" the day of birth. For the preparation of endogenous Na^+, K^+-ATPase inhibitor (endobain E), Wistar male adult rats (35–40 days old) 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.

Determination of [^{3}H]-Inositol Phosphates (IPs)

A procedure based on described methods [21, 22] with modifications, was performed. Cerebral cortices of three neonatal rats were processed for each experimental procedure. Pooled tissue was placed on ice on a Petridish with gassed Krebs-Henseleit buffer containing (mM): NaCl, 120; KCl, 4.7; $CaCl_2$, 1.3; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25 and glucose, 11.7, equilibrated to pH 7.4 with O_2/CO_2 (95:5); tissue was lightly minced [18] and prisms dispersed at 10% (w/v) in the same buffer. Samples were incubated in bulk at 37°C for 1 h under gentle shaking with an intermediate change of buffer, followed by 60 min incubation with [^{3}H]*myo*inositol (6 μCi/ml; final concentration 3×10^{-7} M); thereafter, samples were washed four times every 10 min with fresh buffer replaced under O_2/CO_2 (95:5). Fifty microliters of packed prisms containing 1.2 ± 0.29 mg protein (mean values ± SEM, $n = 20$) were transferred to tubes with 0.24 ml of the same buffer which contained LiCl (7.5 mM final concentration, with NaCl iso-osmotically reduced), and the indicated additions to 0.3 ml final volume. To test drug effect, prisms were incubated for 0 or 30 min in the presence or absence of SR 48692 dissolved in DMSO 10% (v/v); then, ouabain or endobain E were added and incubation proceeded under O_2/CO_2 (95:5) with shaking at 37°C for 20 or 60 min, as indicated in the figure legends. Corresponding controls with redistilled water or 10% DMSO were processed. Prism samples preloaded with [^{3}H]*myo*inositol were processed throughout without additions (in triplicate) to determine basal IPs accumulation.

Incubations were stopped by the addition of 940 μl chloroform:methanol (1:2), followed by chloroform (310 μl) and redistilled water (310 μl) to separate phases. Tubes were vortex-mixed for 15 s, then centrifuged at 1,000*g* for 10 min to facilitate phase separation. Radiolabeled IPs were separated from inositol by ion-exchange chromatography using small columns containing 0.5 ml of a 50% slurry AG 1-X8 resin in the formate form. Upper phase aliquots (750 μl) diluted to 3 ml with redistilled water were added to the resin suspension, centrifuged, and washed 4 times with 3 ml of *myo*inositol (5 mM). [^{3}H]-IPs

were eluted with 1 ml of 1 M ammonium formate/0.1 M formic acid; 800 µl of this eluate were added to 10 ml of OptiPhase "Hisafe" 3 and counted in a Tracor Analytic scintillation spectrometer with 30% efficiency.

The relatively long time of incubation (20–60 min) did not allow to measure the accumulation of the more polar hydrolysis products inositol-1,4-diphosphate and inositol-1,4,5-triphosphate, since changes in these compounds are detectable at very short times after addition of drugs to the incubation buffer [23]. However, since the concentration of ammonium formate employed allows the recovery of all IPs, the term IPs instead of IP_1 (inositol monophosphate) was used.

In all experiments, aliquots containing the same amount of brain tissue (that is, similar intra experimental protein content) were processed, which allowed the comparison between basal condition and treatment in the same sample. Accumulation of [^{3}H]-IPs was thus expressed as the percentage of basal value (without additions).

Preparation of II-E Fraction (Endobain E)

Adult male rats were used. Peak I and II fractions from rat cerebral cortex were prepared as previously described [14, 15]. Thus, for each preparation, cerebral cortices from 5 rats were pooled, homogenized at 25% (w/v) in redistilled water, and centrifuged at 100,000*g* for 30 min in a 70.1 rotor of an L8-Beckman ultracentrifuge. A 5-ml supernatant sample (brain soluble fraction) was taken to pH 7.4 with 0.1 M NH_4HCO_3, loaded on a Sephadex G-10 column (1 × 20 cm) and a single 11-ml fraction collected. This filtrate was then applied to a column packed with Sephadex G-50 (1.8 × 25 cm). For gel equilibration and elution, 0.01 M NH_4HCO_3 was used. Fractions of 1.4 ml each at a flow rate of 0.3 ml per min were collected in a Gilson Fraction Collector 202. The absorbance profile was recorded at 280 nm. Peaks I and II were made up with the five fractions presenting maximal UV absorbance. Peak I was made up with five fractions from 18 to 22 but not used in this study; peak II was made up with five fractions from 49 to 53. Peak II was adjusted to pH 2.0 with 2 M HCl, lyophilized and stored at −20°C. The following day, peak II samples were processed by anionic exchange HPLC on a Synchropak AX-300 column, 250 × 4.6 mm (Synchrom Inc., Lafayette, IN, USA), and eluted at a flow rate of 0.5 ml per min with a 20-min gradient from 0.001 to 0.010 M NH_4HCO_3 to separate fractions II-A to II-H. Fractions were collected by monitoring of absorbance curves at 230 nm; II-E was collected at 12–13 min, then lyophilized, and used within 20 days [13]. Lyophilized II-E samples were dissolved in 0.006 M HCl and immediately prior to assay neutralized with 0.2 M Tris base solution. Hereafter, II-E fraction is termed *endobain E*.

Protein Measurement

Protein was determined by the method of Lowry et al. [24] using bovine serum albumin as standard.

Results

Labeling of inositol phosphates (IPs) in brain prisms of neonatal rats was studied after incubation with [^{3}H]*myo*inositol in the presence or absence of ouabain or endobain E with and without NTS1 receptor antagonist SR 48692.

After 60-min prism incubation, basal [^{3}H]-IPs accumulation was 6,218 ± 717 dpm per mg protein (mean values ± SEM from 10 experiments).

IPs accumulation values reached roughly 500% with 1×10^{-4} M ouabain in comparison with basal values (100%). In order to analyze potential involvement of NTS1 receptor in PI hydrolysis stimulation by ouabain, experiments were carried out under several conditions. Prisms were preincubated for 0 min or 30 min in the absence or presence of $6{,}7 \times 10^{-4}$ M SR 48692, then ouabain was added to reach 1×10^{-4} M concentration and incubation proceeded for 60 min.

PI hydrolysis stimulation by ouabain *versus* basal was 500% or 860% if preincubation was omitted or lasted 30 min, respectively. In each case, these values were diminished roughly 50% by the presence of SR 48692 (Fig. 1a and b).

Previous studies demonstrated that the extent of the incubation period was critical for neurotensin effect on PI hydrolysis [5]. Thus, other series of experiments were performed by reducing the incubation period to 20 min (instead of 60 min). After 20-min prism incubation, basal [^{3}H]-IPs accumulation was 2,026 ± 1218 dpm per mg protein (mean values ± SEM from 4 experiments). If the preincubation period was omitted, ouabain increased up to 290% the PI hydrolysis, a value which was reduced 23% by the presence of SR 48692 (Fig. 2a). When preincubation lasted 30 min, ouabain enhanced PI hydrolysis to 410%, a value reduced 32% with SR 48692 (Fig. 2b).

To test the effect of SR 48692 on PI turnover stimulation by endogenous ouabain-like substance endobain E, experiments were run after 0 min or 30 min preincubation with and without SR 48692. After 60-min prism incubation, basal [^{3}H]-IPs accumulation was 1,296 ± 67 dpm per mg protein (mean values ± SEM from 5 experiments). Results obtained after 60 min incubation indicated that 1 mg/µl endobain E enhanced IPs accumulation to 190%, a value reduced roughly 40% by the presence of SR 48692 (Fig. 3a). In the case of a 30 min preincubation, followed by 60 min incubation, IPs accumulation values were 250%, dropping approximately 30% with SR 48692 (Fig. 3b).

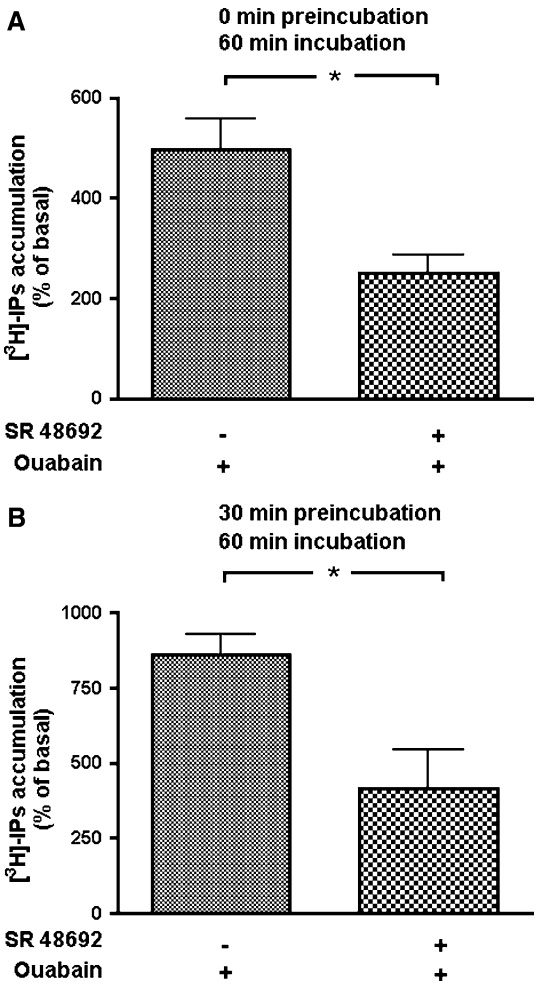

Fig. 1 IP accumulation after 60 min incubation of cerebral cortex prisms obtained from neonatal rats in the presence of ouabain and NTS1 receptor antagonist SR 48692. Prisms were preincubated 0 min or 30 min with or without 10^{-4} M SR 48692, then ouabain was added to reach 10^{-4} M concentration, and incubation proceeded for 60 min. Samples were processed for the assay of IPs. Results are expressed as percentage of basal IP accumulation carried out without additions. Data are mean values (± SEM) from 3 experiments performed in triplicate. (**a**) 0 min preincubation; (**b**) 30 min preincubation. * $P < 0.05$ by Student's t test

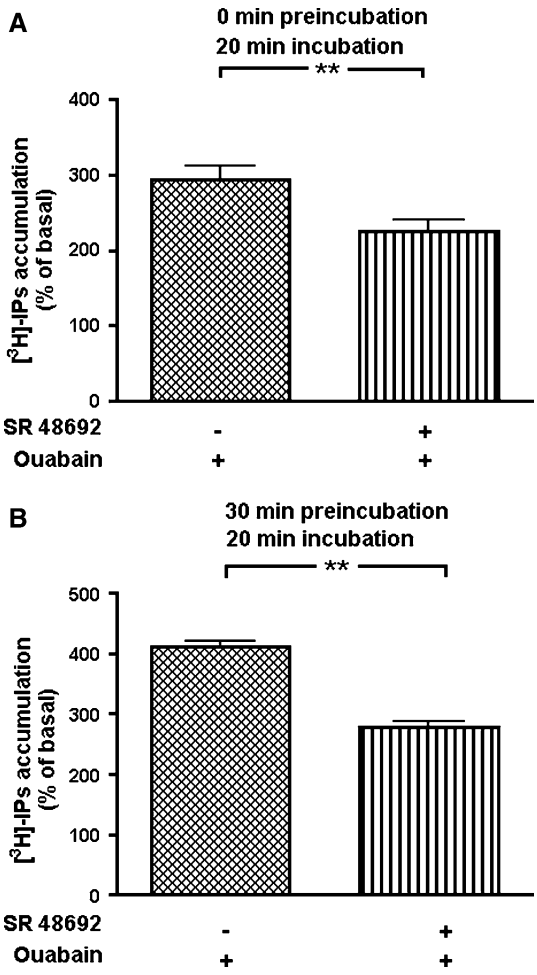

Fig. 2 IP accumulation after 20 min incubation of cerebral cortex prisms obtained from neonatal rats in the presence of ouabain and NTS1 receptor antagonist SR 48692. Prisms were preincubated 0 min or 30 min with or without 10^{-4} M SR 48692, then ouabain was added to reach 10^{-4} M concentration, and incubation proceeded for 20 min. Samples were processed for the assay of IPs. Results are expressed as percentage of basal IP accumulation carried out without additions. Data are mean values (± SEM) from 3–6 experiments performed in triplicate. (**a**) 0 min preincubation; (**b**) 30 min preincubation. ** $P < 0.02$ by Student's t test

In the case of 20-min prism incubation, basal [^{3}H]-IPs accumulation was 1,177 ± 107 dpm per mg protein (mean values ± SEM from 3 experiments). In the condition of 0 min preincubation followed by 20 min incubation with endobain E, IPs accumulation reached 170%, a value reduced 10% with SR 48692 (Fig. 4a). In the case of 30 min preincubation followed by 20 min incubation, IPs accumulation was 410%; this value decreased roughly 30% with SR 48692 (Fig. 4b).

In the present experiments SR 48692 was dissolved in 10% DMSO. Control experiments were therefore performed, to observe that neither SR 48692 solution in DMSO nor the solvent DMSO alone was able to alter PI hydrolysis (data not shown).

Discussion

Previous studies showed that inhibition of Na^+, K^+-ATPase leads to PI hydrolysis enhancement in rat brain (see Introduction). Herein we tested whether high-affinity neurotensin (NTS1) receptor is involved in this effect. To inhibit the enzyme, ouabain and endobain E, an endogenous Na^+, K^+-ATPase inhibitor, were employed. Results indicated that, at least partially, NTS1 receptor participates in PI turnover increase when Na^+, K^+-ATPase activity is inhibited.

The ability of ouabain, the specific and selective Na^+, K^+-ATPase inhibitor, to enhance PI turnover in brain has already been described, an effect markedly higher in

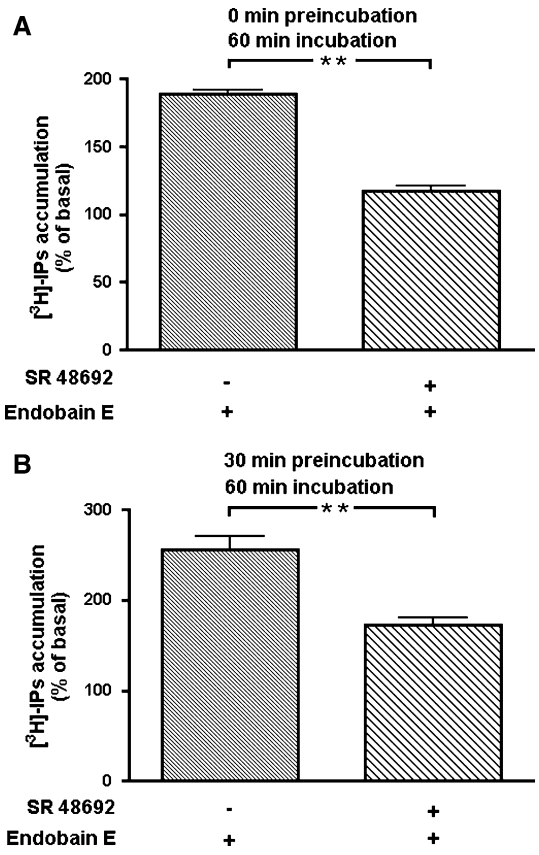

Fig. 3 IP accumulation after 60 min incubation of cerebral cortex prisms obtained from neonatal rats in the presence of endobain E and NTS1 receptor antagonist SR 48692. Prisms were preincubated 0 min or 30 min with or without 10^{-4} M SR 48692, then endobain E was added to reach 1 mg original tissue per ml, and incubation proceeded for 60 min. Samples were processed for the assay of IPs. Results are expressed as percentage of basal IP accumulation carried out without additions. Data are mean values (± SEM) from 5 experiments performed in triplicate. (**a**) 0 min preincubation; (**b**) 30 min preincubation. ** $P < 0.02$ by Student's t test

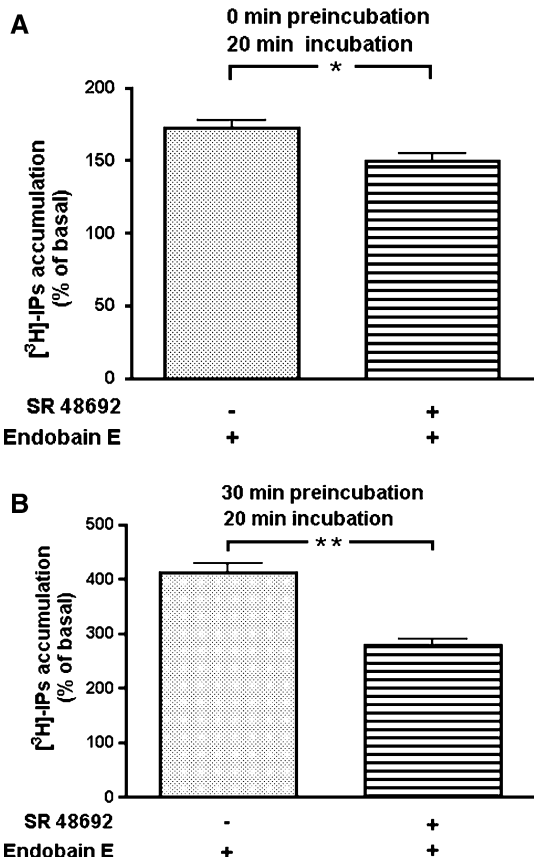

Fig. 4 IP accumulation after 20 min incubation of cerebral cortex prisms obtained from neonatal rats in the presence of endobain E and NTS1 receptor antagonist SR 48692. Prisms were preincubated 0 min or 30 min with or without 10^{-4} M SR 48692, then endobain E was added to reach 1 mg original tissue per ml, and incubation proceeded for 20 min. Samples were processed for the assay of IPs. Results are expressed as percentage of basal IP accumulation carried out without additions. Data are mean values (± SEM) from 3–5 experiments performed in triplicate. (**a**) 0 min preincubation; (**b**) 30 min preincubation. * $P < 0.05$; ** $P < 0.02$ by Student's t test

neonatal than in adult brain [19, 20]. With the endogenous ouabain-like substance termed endobain E [12, 25] an increase in PI turnover was likewise observed, an effect recorded in neonatal but not in adult rat brain [20]. With this background, the present study was carried out in neonatal brain prisms to test potential involvement of NTS1 receptor.

PI turnover is increased by activation of several neurotransmitter receptors, as occurs with carbachol, a cholinergic muscarinic receptor agonist [3]. Following an experimental schedule similar to that employed herein, PI turnover was previously studied in the presence of carbachol, neurotensin and NTS1 antagonist SR 48692. Results indicated that SR 48692 or SR 48692 + neurotensin significantly diminish IP accumulation induced by carbachol. However, the single addition of the peptide fails to alter basal IP accumulation. Taken jointly, results suggested that endogenous neurotensin may be involved in this effect [7].

To test potential participation of NTS1 receptor in PI hydrolysis increase by Na^+, K^+-ATPase inhibition, experiments we run under several conditions. Results obtained indicated that SR 48692 invariably diminished PI turnover increase produced by Na^+, K^+-ATPase inhibition by ouabain or endobain E. Blocking effect of SR 48692 was higher when sample incubation with Na^+, K^+-ATPase inhibitors lasted 60 min *versus* 20 min. The blocking effect was never higher than 50%. In the present work exogenous neurotensin was not added during prisms incubation, and therefore, the effect of SR 48692 would be most likely attributable to the interaction of endogenous neurotensin with its receptor. This finding is in line with previous results indicating that endogenous neurotensin is involved in PI hydrolysis increase by carbachol [7].

It could be hypothesized that a change in endogenous neurotensin occurs due to the presence of SR 48692. In the case of endogenous neurotensin contributing to PI hydrolysis, SR 48692 should decrease basal PI hydrolysis. This seems not to be the case, since our results from control experiments carried out in the presence of SR 48692 (dissolved in 10% DMSO) indicated that the antagonist alone failed to alter basal PI hydrolysis (data not shown). Therefore, the hypothesis that present results involve an alteration of endogenous neurotensin seems untenable. Instead, it seems that SR 48692 has an effect only when PI hydrolysis is enhanced by the presence of a stimulator, i. e., by a Na^+, K^+-ATPase inhibitor (present findings) or by a cholinergic muscarinic agonist like carbachol [7].

It has been demonstrated that phosphoinositide hydrolysis may be stimulated by ouabain and that such effect is significantly higher in neonatal (7 day-old) than in adult rat cerebral cortex [19, 20]. Similar data were recorded with endogenous Na^+, K^+-ATPase inhibitor endobain E [20]. It is worth mentioning that mRNA NTS1 is present at birth and peaks at day seven [26–28] and that both neurotensin and NTS1 receptor peak at 5–10 days after birth [29]. On the other hand, the activity of brain Na^+, K^+-ATPase increases during development [30–32] due to enzyme accumulation [33–35]. It is possible that the greater IP accumulation in neonatal could be due to the relatively lower activity of the sodium pump. It seems that in order to enhance the PI turnover, a lower pump activity is necessary, as in the presence of ouabain. The finding that in adult brain PI turnover stimulation is dramatically lower or nule in the presence of ouabain and endobain E, respectively, may be related to a lower expression of NTS1 receptor.

Present results showed that SR 48692 decreased the effect of Na^+, K^+-ATPase inhibitors on PI turnover. It may be hypothesized that in neonatal brain a higher effect is recorded due to the relatively higher expression of NTS1 receptor versus that of Na^+, K^+-ATPase. It is tempting to speculate that interaction between NTS1 receptor and Na^+, K^+-ATPase exert a regulatory role which would be more operative at early steps of brain development.

Present findings showing that PI hydrolysis enhancement is reduced by SR 48692, indicate that they could be in part due to the activation of neurotensinergic system. Since the NTS1 antagonist decreased partially (not totally) PI turnover increase, other classical neurotransmitter or peptidergic systems may likewise be involved in this effect. Regarding the mechanism (s) underlying PI turnover stimulation by ouabain and endobain E, the participation of ionotropic glutamate receptors in endobain E- and ouabain-induced phosphoinositide hydrolysis seems untenable, since antagonists dizocilpine and CNQX are unable to inhibit these effects [36]. With respect to a direct activation of glutamate metabotropic receptors (mGluRs), using Cd^{2+} in PI hydrolysis experiments [37, 38], the involvement of such receptor type in ouabain effect was advanced [36]. The antagonist for group I mGluRs L-AP3 is able to decrease endobain E effect whereas only a trend to decrease ouabain effect is recorded. On the other hand, ouabain effect is reduced to 50% employing MCPG (a competitive antagonist for group I mGluRs), whereas no blockade is observed with endobain E. In addition, MPEP (a selective mGluR5 antagonist) partially reduces ouabain and endobain E responses while the selective mGluR1 antagonist LY367385 shows no activity at all [36]. These evidences indicate the involvement of mGluR5 in PI hydrolysis stimulation by both endobain E and ouabain in neonatal rat brain, in spite of dissimilar response to tested antagonists.

Taken jointly, findings suggest that besides glutamatergic metabotropic receptors, NTS1 receptor is likewise involved in ouabain and endobain E effect on IPs accumulation in neonatal brain. The blockade of NTS1 receptor seems insufficient to totally prevent PI metabolism stimulation by inhibiting the sodium pump.

Neurotensin inhibits synaptosomal membrane Na^+, K^+-ATPase activity, an effect fully blocked by SR 48692, indicating the involvement of NTS1 receptor [39, 40]. Na^+, K^+-ATPase activity is crucial in the normal cell cycle and differentiation of the nervous system [41, 42]. Recent studies suggest that, in addition to functions previously described, Na^+, K^+-ATPase may act as a signal transducer and activator of gene transcription [43, 44]. There is sufficient evidence to support the idea of Na^+, K^+-ATPase contributing as a signal transducer. The possibility that the signaling enzyme is concentrated in a separate pool on the plasma membrane has been advanced. The enzyme concentrates in caveloae/rafts and a potential interaction between Na^+, K^+-ATPase and caveolins was proposed [45–47]. Many signaling molecules and membrane receptors are dynamically associated with caveolae, mainly through their interactions with caveolins [48–50]. Caveolins stabilize caveolae and modulate signal transduction by attracting signaling molecules to caveolae and regulating their activity [50]. Interestingly, in kidney cells a large proportion of the total cellular phosphatidylinositol 4,5-bisphosphate resides in detergent-insoluble lipid domains enriched in caveolin [51]. Phosphatidylinositol 4,5-bisphosphate is highly compartmentalized within cells and caveolae are the primary site of agonist-stimulated phosphatidylinositol 4,5-bisphosphate turnover [52]. The observation that the inositol trisphosphate receptor is also concentrated in this domain [53] indicates that both the production of and the response to inositol trisphosphate is highly compartmentalized within cells. These findings point to a signaling function of Na^+, K^+-ATPase in nerve ending membranes where the enzyme is concentrated [54].

It should be recalled that NTS1 receptor is one of the G protein-coupled receptors [4]. The discovery of this receptor type dimerization has disclosed a new level of molecular cross-talk between signaling molecules and may define a general mechanism that modulates the function of G protein-coupled receptors under both physiological and pathological conditions [55]. Oligomerization of the receptor and interaction with other receptors or cellular proteins may occur. In this sense, it might be hypothesized that heterodimerization between NTS1 with Na^+, K^+-ATPase or with some of its subunits may take place. Another possibility is that potential dimerization between NTS1 with Na^+, K^+-ATPase may lead to lower PI turnover stimulation and/or that dimerization might change quali- or quantitatively during development.

Since endobain E had a similar effect to ouabain on PI turnover, and given the postulate that Na^+, K^+-ATPase seems to act as a signal transducer, it is tempting to speculate that endobain E behaves as a physiological inducer of this signaling system.

To summarize, present results suggest the participation of NTS1 receptor in PI hydrolysis stimulation when Na^+, K^+-ATPase is inhibited. Since PI hydrolysis enhancement is only partially reduced by SR 48692, then other systems besides the neurotensinergic one must also be involved in this effect.

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