INHIBITORY EFFECT OF NEUROTENSIN ON HIGH AFFINITY $^3$H-OUABAIN BINDING TO CNS MEMBRANES

Rosin C., López Ordieres MG, Rodríguez de Lores Arnaiz G. *

Inst Biol Cel y Neuroc “Prof. E. De Robertis”, Fac Med, y Cátedra de Farmacol, Fac Farm y Bioq, UBA. Paraguay 2155, 1121-Buenos Aires, Argentina.

INTRODUCTION
Neurotensin is a peptide widely distributed through the gastrointestinal tract and the central nervous system. Neurotensinergic system interacts with other neurotransmitter systems, including dopaminergic, cholinergic, serotonergic, opioid and aminoacidergic systems, among others (1).

Neurotensin binds to a group of receptors (2, 3). 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 (3). 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 (4). It has been shown that neurotensin inhibits the activity of synaptosomal membrane Na$^+$, K$^+$-ATPase, this effect most likely involves NTS1 receptor, because it is blocked by antagonist SR 48692 (5). 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 determined in the presence of neurotensin or NTS1 antagonist SR 48692 as well as after the administration of the later.

MATERIALS AND METHODS
□ Lots of six rats were administered i.p. with 100 µg/Kg, 250 µg/Kg SR 48692 or with vehicle (0.01% Tween 80 in saline). Thirty min later, animals were sacrificed, cerebral cortices harvested, homogenized (10% W/V) in ice cold 0.32 M sucrose (pH 7.4) and subjected to differential centrifugation to obtain the membrane fractions.
□ Stereotaxis: Wistar rats (250-300g) were anaesthetized with 300 mg/Kg chloral hydrate and injected into the lateral cerebral ventricle with neurotensin 3, 10 or 30 µg/10 µl, or saline (control) at a rate of 1 µl / min with a Hamilton syringe. Sixty min later animals were decapitated and cerebral cortex harvested to isolate membrane fractions (6).
□ $^3$H-Ouabain binding was carried out by a filtration assay using 45 nM $^3$H-ouabain, with or without neurotensin and/or SR 48692 to evaluate Na$^+$, K$^+$ - ATPase α3 isoform (7, 8).

RESULTS
Results showed that neurotensin and NTS1 receptor antagonist SR 48692 diminished high affinity $^3$H-ouabain binding to cerebral cortex membranes in a dose-dependent manner. The simultaneous addition of both substances produced a synergic action. Neurotensin increased Kd without changes in Bmax, indicating a competitive type inhibition. The administration of either neurotensin or the antagonist, invariably decreased $^3$H-ouabain binding. Injection of SR 48692 failed to modify neurotensin inhibitory effect on ouabain binding.

CONCLUSIONS
Results suggested an inhibitory action of neurotensin and SR 48692 on $^3$H-ouabain binding and that SR 48692 did not prevent neurotensin effect on ouabain binding. It is concluded that neurotensin is able to modulate $^3$H-ouabain binding, an effect which hardly involves NTS1 receptor.

REFERENCES

* Corresponding author. E-mail: grodrig@ffyb.uba.ar
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