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Original articles

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THE DIHYDROPIRIDINES MODULATE NEUROTENSIN INOTROPIC ACTION PARADOXICALLY

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The interaction of neurotensin and calcium channel modulators is examined in isolated electrically driven guinea pig left atrial appendages. Left guinea pig atria exposed to diltiazem become desensitised to neurotensin. There is no significant influence of verapamil pretreatment on the neurotensin inotropic action. Nifedipine pretreatment causes increase in the inotropic response of guinea pig atria to neurotensin. The regression line of neurotensin after pretreatment with nifedipine compared to the regression line of neurotensin alone has higher slope and is shifted to the left. The ED_{50} of neurotensin after nifedipine pretreatment compared with the ED_{50} of neurotensin alone resultes in potency ratio of 2.24. Bay K8644 significantly decreases the inotropic effect of ED_{100} of neurotensin. Results suggest that: (1) the mechanism of interaction of calcium channel modulators and neurotensin in the atrium does not depend on the calcium influx through calcium channel nor does it on the calcium channel itself; (2) the interaction of nifedipine and neurotensin is possibly dependent on dihydropiridine receptors, (3) the dihydropiridine binding site, possibly different from voltage-sensitive calcium channel, is somehow involved in the neurotensin action in guinea pig atria.

Key words: *neurotensin, calcium channel modulators, guinea pig isolated atria.*

INTRODUCTION

Neurotensin, a natural tridecapeptide of nervous and intestinal origin (1, 2) exerts numerous actions in the cardiovascular system of mammals. The peptide has been found to produce a decrease in blood pressure in rats, rabbits, pigs,

goats and dogs (3). Pressor and biphasic pressor-depressor responses are observed in guinea pigs, woodchucks, sheep and cats (3). Triphasic neurotensin effects on the rat blood pressure are also reported (4). Although the physiological role of neurotensin in the cardiovascular system of humans remains unclear, it has been demonstrated to be a circulating hormone (5) with plasma levels varying, e.g. in the response to food ingestion (6). Hemodynamic actions of neurotensin are thought to be mediated by histamine, serotonin, and catecholamines (3). Nevertheless the direct action of neurotensin in cardiac muscle is also reported. The peptide exerts positive inotropic and chronotropic effects in isolated guinea pig and rat atria (7). Quirion and co-workers have demonstrated that action to be mediated by specific neurotensin receptors presumably located in atrial cell membranes (8).

It is shown in the adenocarcinoma HT29, cell line that neurotensin binds to high-affinity receptors, and that receptors occupancy leeds to the inositol phosphate formation. The properties of the effects of Na^+ and GTP on neurotensin-receptor interactions are characteristic of those receptors that interact with G-proteins (9). In HT29 cells the well defined neurotensin receptor-inositol phosphate intracellular Ca^{2+} pathway is described (10). The same pathway has been identified in neuroblastoma x glioma hybrid NG 108-15 cells (11). In both models an intracellular Ca^{2+} concentration increase as well as an increase in the formation of inositol phosphates are not altered by the absence of extracellular Ca^{2+} (11, 12).

On the other hand neurotensin effects in gastrointestinal smooth muscle depend on extracellular calcium concentration (13, 14, 15). Huidobro-Toro and Kullak, in the study on the smooth muscles of the rat fundus, have concluded that neurotensin activates specific excitatory receptors probably located on the cell membrane. They suggest that these receptors are somehow related to a voltage-dependent calcium channel, sensitive to verapamil (16). Interestingly, inhibition of ileal muscle contraction by neurotensin is also blocked by verapamil and nitrendipine (17). Generally, biological activities of neurotensin in gastrointestinal muscles of different species — the contracting effect in stomach fundus strips, the contraction of circular duodenal muscle, the relaxant effect in longitudinal duodenal muscle, the relaxation of jejunum and the stimulation of circular colonic muscle — are all blocked by calcium channel blockers and enhanced by calcium channel activators (13—19).

Although neurotensin receptors have been identified in guinea pig atria since 1980 (8), we know nothing of the role of calcium channels in the neurotensin action in myocardium.

The aim of the present study is to assess calcium channel modulators influence on neurotensin inotropic effects in the cardiac muscle.

MATERIALS AND METHOD

Guinea pig left atrial appendages were used. Male guinea pigs weighing 400—500 g were sacrificed by decapitation. Immediately after that the left atrial appendages were dissected and placed in oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution of the following composition (mM): NaCl 118.0, KCl 4.70, $CaCl_2$ 2.52, $MgSO_4$ 1.64, $NaHCO_3$ 24.88, KH_2PO_4 1.18, Glucose 11.1 (pH 7.4). The bath was maintained at 37°C.

After a short period of initial incubation the tissues were mounted under a resting tension of approximately 15 mN. In those settings the tissues were equilibrated for 45—60 minutes before the administration of drugs. The tissues were driven with platinum field electrodes applying square-wave stimuli of the width of 5 ms, the frequency 2.5 Hz. The amplitude of stimulus was 150% of the threshold for the given preparation. Before being challenged with drugs the preparations were stimulated for 5—8 minutes, which time was sufficient to achieve stable amplitude of contractions. The effect of a drug was measured 2 minutes after it had been added to the bath. When examining interactions the tissue had been pretreated with calcium channel blocker or activator for 2 minutes, and next neurotensin was added. The doses of calcium channel antagonists and agonists were adjusted to cause 30—40% decrease or increase in tissue contractility, respectively. Doses established that way were as follows: verapamil $10^{-4.8}$ M, diltiazem $10^{-4.7}$ M, nifedipine $10^{-6.5}$ M, Bay K8644 $10^{-6.5}$ M.

Drugs used were supplied by: Sigma — neurotensin, Serva — verapamil, Orion — diltiazem, Bayer — nifedipine. Bay K8644 was a generous gift of Bayer.

8—12 preparations were used to assess each effect. The effects were presented as the percent of the change in contraction force.

Data were evaluated statistically using the Mann-Whitney nonparametric test to compare effects at particular dose levels. Where needed the linear regression analysis was performed. We constructed regression lines for theoretically linear dose-response curve fragments (between 20% and 80% of maximal effect). The regression lines were compared using covariance analysis and multiple regression analysis. The values of ED_{50} were calculated and compared using potency ratio.

RESULTS

The dose-response relationship for neurotensin inotropic action in electrically driven guinea-pig left atria appendages was obtained as the startpoint for studying neurotensin and calcium channel modulators interaction (*Fig. 1*). The neurotensin ED_{50} equalled $10^{-7.5}$ M (*Fig. 2*).

Left guinea pig atria exposed to diltiazem became desensitized to neurotensin. There was no significant influence of verapamil pretreatment on the neurotensin inotropic action (*Fig. 2*).

Nifedipine pretreatment caused an increase in the inotropic response of guinea pig atria to neurotensin (*Fig. 1*). The regression line of neurotensin after pretreatment with nifedipine compared to the regression line of neurotensin alone had higher slope (49.4 vs. 30.0; $p < 0.05$) as assessed by covariance analysis and was shifted to the left (431.4 vs. 257.5; $p < 0.05$) as assessed by multiple regression method (*Fig. 2*). The ED_{50} of neurotensin after nifedipine

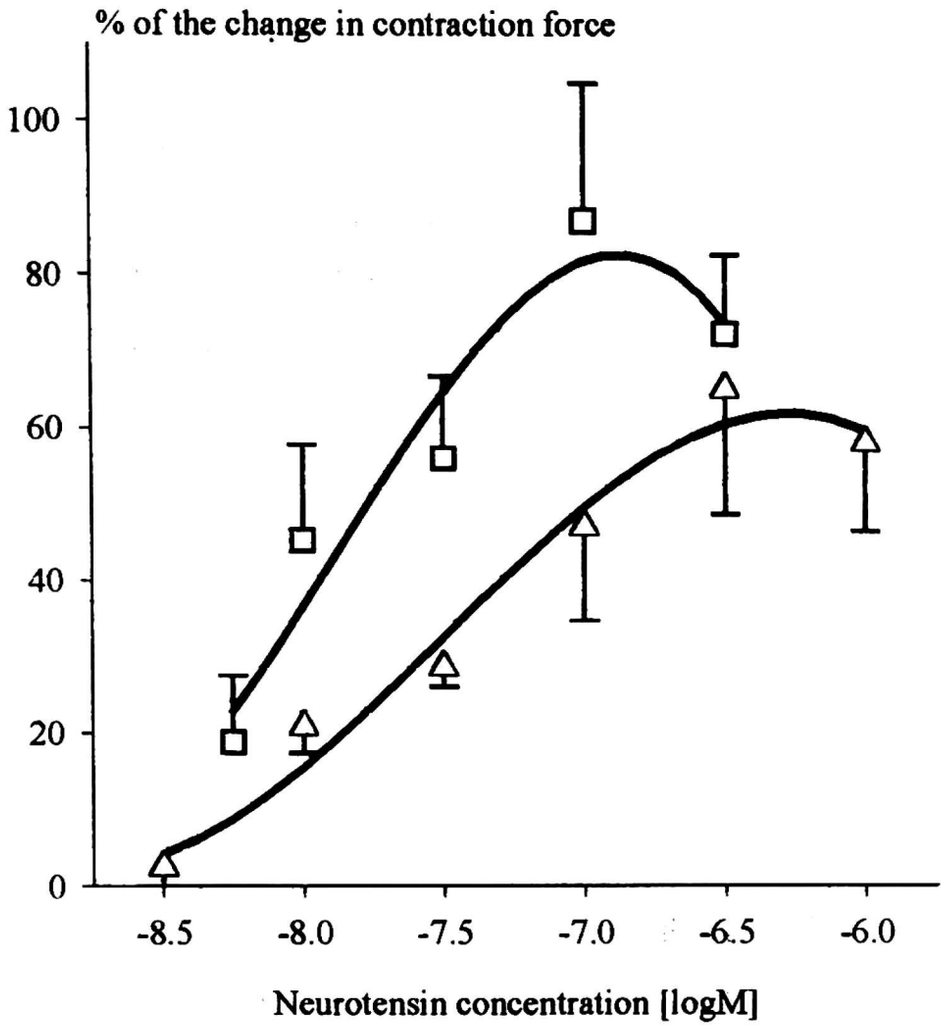

Fig. 1. The dose-response relationship for neurotensin and neurotensin with nifedipine pretreatment in electrically driven guinea-pig left atria appendages (lines fitted by eye). △ — neurotensin, □ — neurotensin with nifedipine pretreatment. Data presented as mean ±S.E.M.; 8—12 measurements for each point.

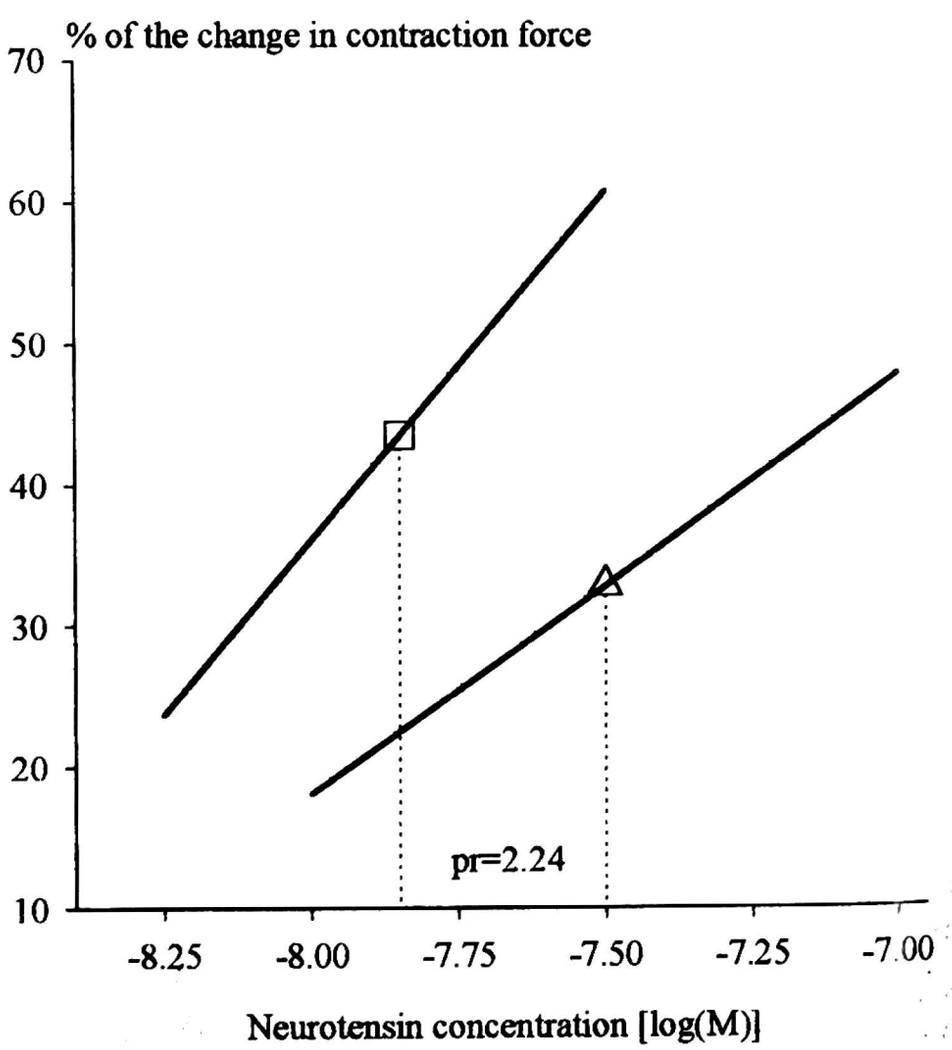

Fig. 2. The regression line for neurotensin after pretreatment with nifedipine compared to the regression line for neurotensin alone. △ — neurotensin, □ — neurotensin with nifedipine pretreatment. Points denote ED_{50} doses. pr — potency ratio. (regression equations: for neurotensin — effect (% force) = 257.499 + 29.9545 * dose (log); [$p < 0.05$, $R^2 = 53.57\%$]; for neurotensin with nifedipine — effect (% force) = 431.381 + 49.4108 * dose (log); [$p < 0.05$, $R^2 = 40.28\%$]).

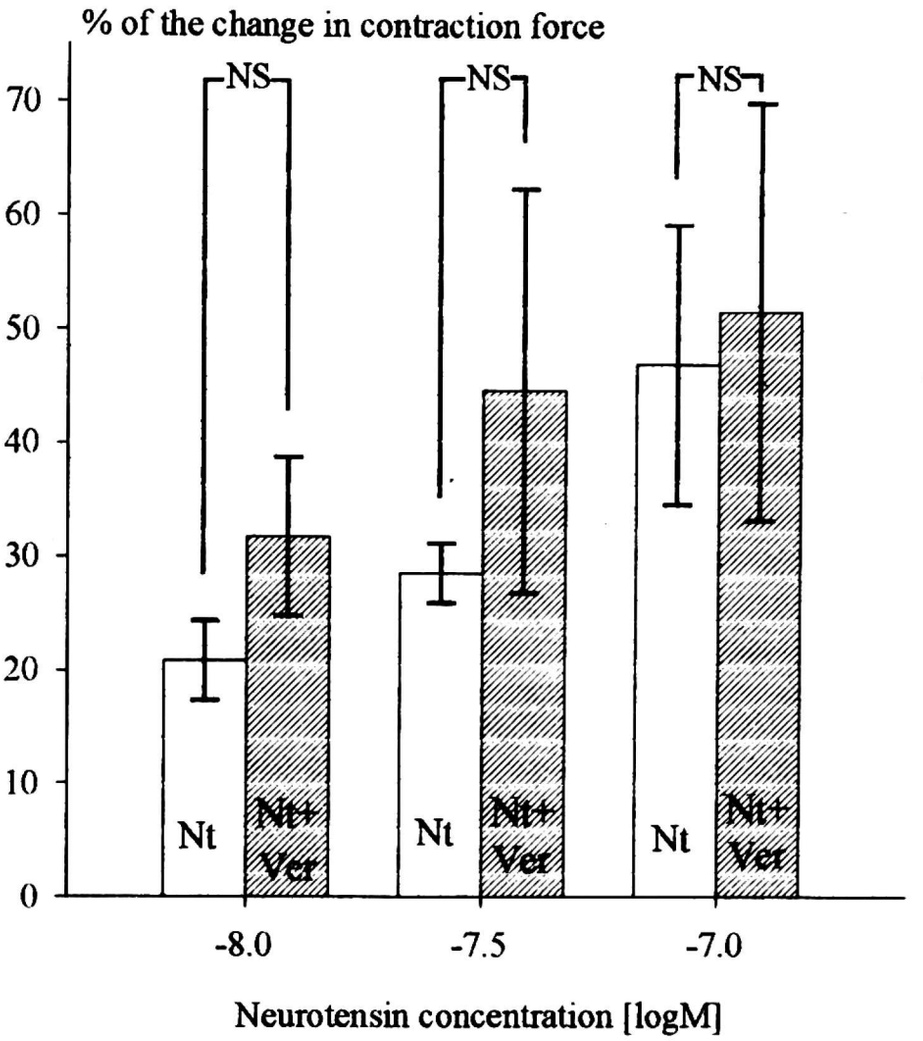

Fig. 3. Inotropic effects of neurotensin after pretreatment with verapamil compared to neurotensin alone. Nt — neurotensin, Nt + Ver neurotensin with verapamil pretreatment. Data presented as mean ±S.E.M.; 8—12 measurements for each point. NS — non significant.

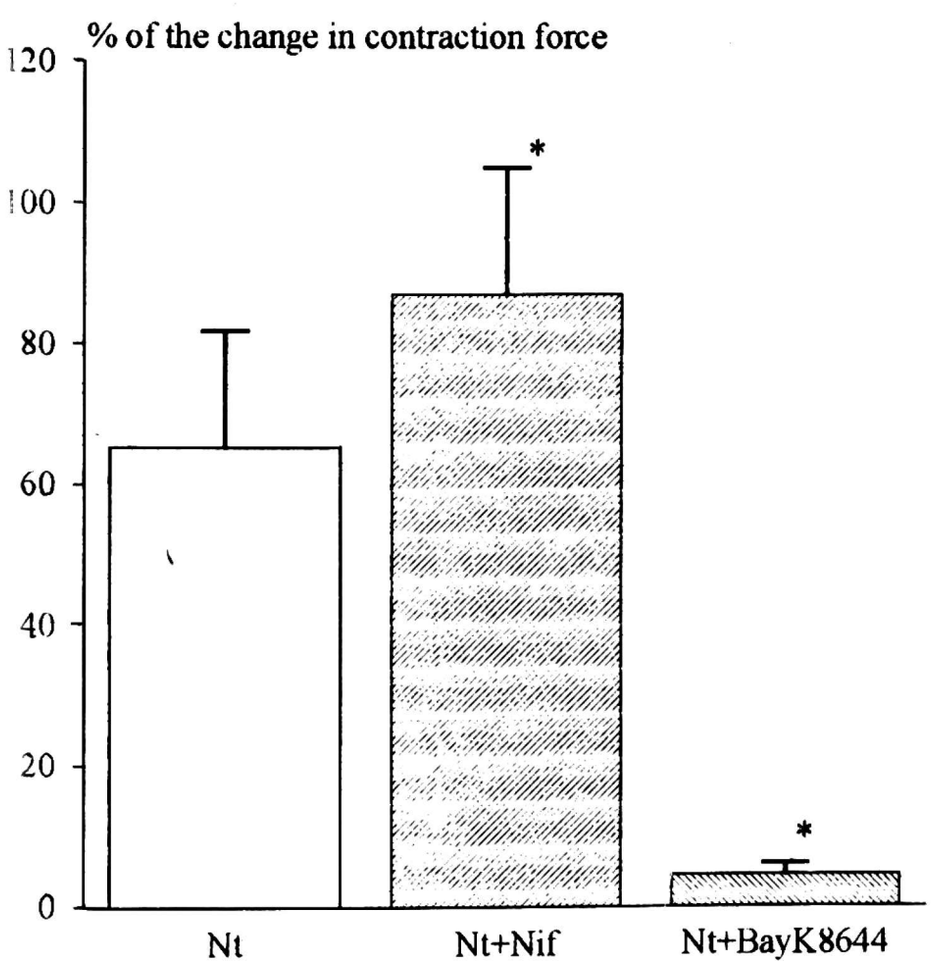

Fig. 4. Influence of nifedipine and Bay K8644 pretreatment on inotropic effect of ED_{100} of neurotensin. Nt — neurotensin, Nt + Nif — neurotensin with nifedipine pretreatment, Nt + Kay K8644 — neurotensin with Bay K8644 pretreatment. Data presented as mean ±S.E.M.; 8—12 measurements for each point. * — $p < 0.05$ vs. neurotensin alone.

pretreatment equalled $10^{-7.85}$ M and compared with the ED_{50} of neurotensin alone resulted in the potency ratio of 2.24 (*Fig. 2*).

Bay K8644 significantly decreased ($p < 0.05$) the inotropic effect of the ED_{100} of neurotensin. The maximal inotropic effect of neurotensin equal $65.3 \pm 12.2\%$ was lowered by the Bay K8644 pretreatment to $4.5 \pm 5.1\%$ (*Fig. 4*).

DISCUSSION

The dose-effect relationship for the neurotensin inotropic action in left guinea pig atria obtained in our study is shifted to the right compared to that obtained by Quirion et al. in 1980. Our experiment has been performed on the electrically driven appendages, whereas Quirion has used spontaneously beating auricles, what can account for observed difference.

One could presume, that the neurotensin action as a positive inotropic agent would depend on the amount of calcium available for the contraction mechanisms. Hence, calcium channel antagonists would uniformly decrease neurotensin inotropic effects in the non-receptor dependent manner, whereas calcium channel agonists should increase them.

Our results are not consistent with that presumption, suggesting that neurotensin and calcium channel modulators interaction in the guinea pig atrial muscle could be receptor mediated. The fact, that neurotensin receptors activation triggers calcium dependent postreceptor mechanisms is well known.

Neurotensin causes the relaxation of longitudinal muscle of the guinea pig stomach, circular muscle of the rat duodenum et ileum and the inhibition of contraction of the canine and rat longitudinal ileal muscle. Receptors associated with the calcium dependent, apamine sensitive potassium channel appear to be responsible for that neurotensin action (15, 17, 18, 20). Mule et al. suggest that these inhibitory receptors are different from excitatory ones (19).

The neurotensin dependent inositol triphosphate pathway leading to the intracellular calcium concentration increase is well defined in HT29 adenocarcinoma cells. The increases in intracellular calcium and cGMP are not altered in the absence of extracellular calcium (9, 12).

On the other hand, the neurotensin induced contraction in gastric and intestinal smooth muscle depends on extracellular calcium (13, 14), suggesting that receptors involved in this action are distinct from those described in the HT29 adenocarcinoma cells. Moreover, all examined calcium channel blockers antagonise contractile responses to neurotensin, whereas Bay K8644, the calcium channel agonist, enhances neurotensin effects. Authors suggest, that the neurotensin receptor is related to voltage sensitive calcium channel, or even that neurotensin acts through that channel (13, 16, 19, 21).

The smooth muscle contraction may be the effect of either pharmacomechanical or electromechanical coupling. The pharmacomechanical coupling depends on cGMP — inositol triphosphate pathway, while the electromechanical coupling requires membrane depolarisation and Ca^{2+} influx through calcium channel for contraction. Although the neurotensin receptor dependent inositol triphosphate pathway is well described and could potentially be involved in the neurotensin contractile action in smooth muscle, the neurotensin induced contractions in gastrointestinal smooth muscles appear to be voltage-sensitive calcium channel mediated (electromechanical coupling). Since pharmacomechanical coupling is unique for smooth muscle, coupling of neurotensin receptors to voltage dependent calcium channel seems even more likely in myocardium.

The above hypothesis cannot account for our results, as we have found that various calcium channel blockers modify neurotensin inotropism in the different way. We can imagine, that in our particular experimental settings diltiazem has blocked and verapamil has not changed the neurotensin inotropism in accordance with described hypothesis. We cannot explain however the positive nifedipine and neurotensin interaction that way. Interestingly the positive inotropic effect of neurotensin has been antagonised by Bay K8644.

These results suggest the possibility, that the mechanism of interaction of dihydropiridine calcium channel modulators and neurotensin in the guinea pig atrium does not depend on the calcium influx through calcium channel. However, the opposite action of nifedipine and Bay K8644 in our experiment suggests, that examined interaction is somehow related to the dihydropiridine binding site.

Our paradoxical results, opposite to what is known of the neurotensin and calcium channel modulators interaction in gastrointestinal muscle, make possible to hypothesise, that dihydropiridine receptors, and not just calcium influx, are in some way involved in the neurotensin action in the atrial tissue. Moreover, the fact, that the interaction is calcium influx independent suggests, that the dihydropiridine binding site involved in the neurotensin action could be different from the one described on the α_1 subunit of the voltage dependent calcium channel, known to be the dihydropiridine receptor (22).

We understand that neurotensin dependent calcium mechanisms can account for the neurotensin inotropic action, and that reducing available calcium by means of calcium channel antagonists can decrease potency of the neurotensin inotropic action. We suggest however, that in guinea pig atria, the dihydropiridine receptor related mechanism is strong enough to overcome the "normal" calcium influx reduction.

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