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Impact and mechanisms of action of neurotensin on cardiac contractility in the rat left ventricle

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Abstract

Using immunoassay measurements, neurotensin was identified in rat ventricular tissue and in coronary effluent samples. Exogenous neurotensin evoked contractile responses in isolated ventricular preparations, which were equivalent in magnitude to those of norepinephrine and histamine, but greater than those for serotonin and angiotensin II. EC_{50} values revealed neurotensin to be as potent as serotonin, but more potent than norepinephrine, histamine and angiotensin II. Structure–activity studies indicated that the contractile effects are attributed to the C-terminal portion of neurotensin. Neurotensin-induced responses were decreased by SR 48692, a specific neurotensin receptor antagonist. Neurotensin elicited an increase in coronary effluent norepinephrine concentrations, and a strong relationship between the magnitude of neurotensin-induced contractile effects and increments in myocardial norepinephrine release were noted. Neurotensin-induced contractile responses were abolished by β-adrenoceptor antagonists, but not by histamine, serotonin or angiotensin II receptor antagonists. In conclusion, neurotensin increases ventricular contractility through stimulation of myocardial norepinephrine release.

Keywords: Neurotensin; Ventricular contractility; Inotropic effect

1. Introduction

Neurotensin is a tridecapeptide originally identified in bovine hypothalami (Carraway and Leeman, 1973) and subsequently found throughout the body, including cardiovascular structures. Importantly, a dense neurotensin-immunoreactive fibre network innervates atrial and ventricular tissue (Forssmann et al., 1982; Reinecke et al., 1982; Goedert et al., 1984; Onuoha et al., 1999a,b). Despite data that indicate a profound inotropic influence of neurotensin on atrial tissue (Quirion et al., 1978, 1980b), studies performed to-date have failed to show effects on ventricular chamber or myocardial systolic function (Rosell et al., 1976; Rigel et al., 1989; Ertl et al., 1993). As the impact of neurotensin on ventricular systolic function is still in question, we sought to explore the possibility that neurotensin elicits physiologically relevant effects on left ventricular contractile function and the potential mechanisms thereof. We assessed whether neurotensin could be identified within ventricular tissue and whether it is released from this tissue. Second, we determined whether neurotensin and its active C-terminal hexapeptide (Quirion et al., 1980a,b; Rioux et al., 1980; Gilbert et al., 1986) elicits increases in left ventricular contractile function under controlled loading conditions and at constant heart rates, and whether these effects could be abolished by a selective neurotensin receptor antagonist. Third, to establish the physiological relevance of any contractile effect noted we compared the efficacy and potency of neurotensin with other known inotropic agents including norepinephrine, serotonin, histamine and angiotensin II. Last, as neurotensin has neuromodulatory actions (Okuma and Osumi, 1982; Kerouac et al., 1984; Rioux et al., 1984; Ertl et al., 1993), we assessed whether the impact of neurotensin is in-part mediated through presynaptic control of myocardial norepinephrine release or through the actions

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of alternative endogenous inotropes, including serotonin, histamine and angiotensin II.

2. Materials and methods

These studies comply with the European Community Guidelines for the Care and Use of Experimental Animals, and were approved by the Animal Ethics Screening Committee of the University of the Witwatersrand (clearance number: 2003/30/3). Male Sprague–Dawley rats weighing 350–500 g were housed in individual cages, and exposed to light by means of an automated system from 07:00 to 19:00 hours. All animals were allowed to acclimatize to the housing conditions for at least 10 days prior to entry into the study.

2.1. Isolated, perfused heart preparations

Inotropic responses were assessed as previously described (Norton et al., 1999). Rats were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (15 mg/kg), a thoracotomy was performed and the heart excised and placed in an ice-cold physiological saline solution. The hearts were retrogradely perfused via the aorta at a constant flow with carefully filtered and warmed (37 °C) physiological solution saturated with 95% O_2 and 5% CO_2. The solution contained (in mM) 118.0 NaCl; 4.7 KCl; 2.5 $CaCl_2$; 25 $NaHCO_3$; 1.2 KH_2PO_4; 1.2 $MgSO_4$; and 10.0 glucose, and had a pH of 7.4. The coronary flow rate was measured by collecting venous effluent and adjusted to achieve a value of 10 ml/min per g of heart weight. To exclude rate-dependent variations in contractility, the cardiac preparations were paced at 330 beats/min via platinum wire electrodes attached to the right atrium and the apex of the left ventricle with the voltage 10% above threshold. In order to record left ventricular developed pressure, the left atrium was trimmed and a water-filled balloon-tipped cannula coupled to a Statham P23 pressure transducer, introduced through the atrioventricular orifice into the left ventricular cavity. Left ventricular developed pressure was calculated as the difference between end-systolic and end-diastolic pressures recorded on a Hellige polygraph. The maximum rates of left ventricular pressure development ($+dP/dt$) and relaxation ($-dP/dt$) were obtained using a differentiator (model 13-4616-71, Gould Instrument Systems, Valley View, OH) with a high-frequency cutoff set at 300 Hz.

2.2. Pharmacological responses

Isolated, perfused heart preparations were allowed to stabilize for 15 min prior to assessing inotropic responses. The substances used in the experiments were dissolved in the same physiological saline solution used to perfuse the hearts and infused just proximal to the aortic cannula by means of a Harvard infusion pump (model 22 M T/W). The rate of infusion was 0.3 ml/min, and the duration of infusion of each concentration of each agent was 60 s. Concentration–response relations were constructed by assessing contractile responses to incremental concentrations of each pharmacological agent. Intervals of at least 5 min were allowed between infusions for left ventricular developed pressures to return to baseline values after a preceding pharmacological exposure. Prior to the infusion of active agents, we ascertained that the vehicle produced no left ventricular contractile responses.

In order to compare the efficacy (maximal inotropic response) and potency (EC_{50}) of neurotensin to other known positive inotropic agents, concentration–contractile response curves were constructed for neurotensin, norepinephrine, histamine, serotonin and angiotensin II. To identify that component of the neurotensin molecule responsible for its contractile effects, concentration–contractile response curves were compared for native neurotensin, C-terminal (8–13) and N-terminal (1–6) fragments of neurotensin, and [D-Trp^{11}]-neurotensin, a neurotensin analogue with a modified C-terminal portion. Hearts were exposed to no more than four concentrations of each agent in the same preparation to avoid the development of tachyphylaxis.

Studies were performed to elucidate whether the contractile effects elicited by neurotensin are neurotensin receptor-mediated and/or explained by the activation of histamine, serotonin, angiotensin II, or norepinephrine receptors. For these studies, the contractile response to a single dose of neurotensin (10^{-8} M) was initially recorded and then the response was again recorded 10 min after infusion of either SR 48692 (10^{-8}–10^{-7} M), a specific neurotensin receptor antagonist (Gully et al., 1993), cimetidine (10^{-7} M), a histamine H_2 receptor antagonist, methysergide (10^{-7} M), a non-selective 5-hydroxytryptamine (5-HT) (5-HT_1, 5-HT_2 and 5-HT_7) receptor antagonist, losartan (10^{-7} M), an angiotensin II type I (AT_1) receptor antagonist, propranolol (10^{-7} M), a non-selective β-adrenoceptor antagonist, or atenolol (10^{-6} M), a selective β_1-adrenoceptor antagonist.

2.3. Neurotensin enzyme-linked immunoassay

Neurotensin concentrations were determined by a competitive-binding enzyme-immunoassay method with a commercially available kit (EIA 421730, Phoenix Pharmaceuticals, Inc., USA) utilizing antibodies with no cross-reactivity to substance P, kinetensin and bombesin, and with only a 0.03% cross-reaction with porcine neuromedin N. Fresh ventricular tissue and coronary effluent samples (10 ml) were frozen in liquid nitrogen and stored at −70 °C. Frozen tissue samples were weighed, then placed in pre-heated 0.1 N acetic acid and boiled for 10 min. The tissue samples were subsequently cooled on ice, homogenized for 2 min in 5 ml of 0.1 N acetic acid, and centrifuged at 13,000 ×*g* for 15 min. Delipidation and precipitation of proteins from both 4 ml of the supernatant obtained from the tissue preparation as well as the coronary effluent was achieved by adding samples to equal volumes of butanol : isopropyl ether and subsequently centrifuging

samples at 8000 ×*g* for 5 min. Pellets were reconstituted in 1 ml of 0.1 N acetic acid and extraction was performed using Sepak columns. Eluates were evaporated and stored at −20 °C overnight. Prior to immunoassay, dried extracts were reconstituted in 250 μl of assay buffer. Duplicate samples were incubated with the specific antibody and biotinylated-labeled neurotensin which was used as a tracer. After removing unbound biotinylated neurotensin by washing, streptavidin-conjugated horseradish peroxidase was added. The amount of neurotensin bound in the antibody sandwich was detected colorimetrically using 3,3′,5,5′-tetramethylbenzidine and hydrogen peroxide. Absorbance was measured on a plate photometer at 450 nm. The minimum detectable concentration of neurotensin was 0.44 ng/ml. Inter- and intra-assay variations were less than 5% and 14%, respectively.

2.4. Myocardial norepinephrine release

To assess whether neurotensin-induced inotropic responses are mediated through stimulation of myocardial norepinephrine release, samples of coronary effluent were collected from isolated heart preparations perfused at constant coronary rates. Coronary effluent was stabilised by adding Na_2EDTA and $HClO_4$ to 10 ml of coronary effluent to achieve a final concentration of 0.01 mol l^{-1} and 0.025%, respectively. Norepinephrine was immediately extracted from 1 ml of coronary effluent using alumina (Sigma) adsorption with a Tris buffer at pH 8.6 eluted with 0.1 M $HClO_4$, stored at −70 °C and concentrations determined using reversed phase, ion-exchange high performance liquid chromatography with electrochemical detection (Ganhao et al., 1991). As all hearts were perfused at the same flow rates per gram of tissue, myocardial norepinephrine release was expressed as the concentration of norepinephrine in the effluent.

2.5. Pharmacological agents

Angiotensin II acetate, 5-hydroxytryptamine hydrochloride (serotonin), histamine dihydrochloride, (±)-arterenol hydrochloride (norepinephrine), neurotensin, fragment (8–13), (±)-atenolol, (±)-propranolol hydrochloride, cimetidine, and methysergide maleate were purchased from Sigma Chemicals (St. Louis, USA). Neurotensin, [D-Trp^{11}]-neurotensin and neurotensin, fragment (1–6), were obtained from ICN Biomedicals Inc. (OH, USA). Losartan was purchased from DuPont Pharmaceuticals (OH, USA). SR 48692 (2-{[1-(7-Chloro-quinolin-4-yl)-5-(2,6-dimethoxy-phenyl)-1H-pyrazole-3-carbonyl]-mino}-adamantane-2-carboxylic acid) was kindly provided by Sanofi Recherche (Toulouse, France).

2.6. Data analysis

Results are expressed as mean ± S.E.M. The magnitude of inotropic responses elicited by pharmacological interventions was expressed as a percent increase in left ventricular developed pressure (Norton et al., 1999). The concentrations of substances that produced 50% of the maximal contractile response (EC_{50}) were determined from regression analysis using logistic sigmoid function curves (log concentration vs. effect) (Norton et al., 1999), and presented throughout as pEC_{50} values ($pEC_{50} = -\log_{10}EC_{50}$). The magnitude of inotropic responses elicited over the range of concentrations of each substance evaluated was assessed by repeated measures ANOVA (analysis of variance), followed by a Tukey–Kramer post hoc test. A comparison of pEC_{50} values and the magnitude of maximal contractile responses was performed using an unpaired Student's *t* test. A paired Student's *t* test was used to compare neurotensin-induced inotropic responses at baseline and after prior infusions of antagonists, as well as coronary effluent norepinephrine concentrations determined at baseline and following neurotensin infusion. Probability values <0.05 were considered to be significant.

3. Results

3.1. Myocardial and coronary effluent neurotensin concentrations

Ventricular tissue contained measurable quantities of neurotensin with concentrations of 10 ± 2 and 13 ± 3 ng/g detected in the left and right ventricles, respectively ($P = 0.38$). The neurotensin concentration in the coronary effluent from isolated perfused heart preparations was 9 ± 6 pmol/ml.

Table 1
Baseline left ventricular developed pressure and $+dP/dt$ values in the different experimental groups used to compare efficacy and potency of neurotensin with other inotropes

Experimental groups	Left ventricular developed pressure, mm Hg	Left ventricular $+dP/dt$, mm Hg/s
Neurotensin, ($n=16$)	94 ± 5	1670 ± 127
Norepinephrine, ($n=13$)	97 ± 9	1733 ± 250
Histamine, ($n=13$)	95 ± 10	1725 ± 300
Serotonin, ($n=11$)	100 ± 7	1884 ± 211
Angiotensin II, ($n=12$)	109 ± 7	2093 ± 184
Neurotensin (8–13), ($n=15$)	105 ± 9	1931 ± 248
Neurotensin (1–6), ($n=7$)	111 ± 10	2181 ± 154
[D-Trp^{11}]-neurotensin, ($n=16$)	104 ± 6	1920 ± 193

No differences were noted in baseline systolic function between the groups. Means ± S.E.M.

3.2. Contractile responses to neurotensin in comparison to other endogenous inotropes

There were no baseline differences in left ventricular developed pressure and $+dP/dt$ values between the different experimental groups of rats (Table 1). Neurotensin evoked potent dose-dependent positive inotropic responses (Figs. 1 and 2). The pEC_{50} value for neurotensin-induced contractile responses was 10.8±0.4 M, and the maximal increase in left ventricular developed pressure achieved at a concentration of 10^{-7} M was 78±5%. The Hill slope of the neurotensin dose–response curve was 0.6±0.1. The positive inotropic response elicited by a 60 s infusion of neurotensin developed rapidly. For example, at a dose of 10^{-7} M, the latency and time-to-peak response was 28±5 and 24±3 s, respectively.

The prominent inotropic effect of neurotensin is demonstrated by comparing its actions with other endogenous inotropes. Indeed, the maximal increase in left ventricular developed pressure in response to neurotensin was the same as that induced by norepinephrine (10^{-5} M, 89±9%, $P=0.3$) and histamine (10^{-3} M, 58±10%, $P=0.1$), and greater than that for both serotonin (10^{-4} M, 51±4%, $P=0.0006$) and angiotensin II (10^{-7} M, 50±9%, $P=0.01$) (Figs. 2 and 3). Therefore, in the isolated rat left ventricle, neurotensin is at least as effective as norepinephrine and histamine and more effective than serotonin and angiotensin II at eliciting contractile responses. Moreover, the mean pEC_{50} value for neurotensin was lower than that for norepinephrine (7.4±0.2 M, $P<0.0001$), histamine (5.5±0.2 M, $P<0.0001$) and angiotensin II (8.8±0.7 M, $P=0.04$), and similar to that of serotonin (11.0±0.4 M, $P=0.73$) (Fig. 3). Thus, in the isolated rat left ventricle, neurotensin is at least as potent as serotonin, but twice as potent as angiotensin II, three times more potent than norepinephrine, and five times more potent than histamine at eliciting positive inotropic responses.

3.3. Contractile responses to components of the neurotensin molecule

Neurotensin (8–13), the C-terminal fragment of neurotensin, produced potent contractile responses in isolated rat left ventricles (Fig. 4A). At the two highest concentrations employed (10^{-6} and $10^{-5.5}$ M), inotropic responses to neurotensin (8–13) were lower as compared to contractile effects elicited by the same concentrations of native neurotensin (10^{-6} M: neurotensin (8–13), 59±3%, native neurotensin, 73±4%, $P=0.02$; $10^{-5.5}$ M: neurotensin (8–13), 58±4%, native neurotensin, 79±3%, $P=0.001$). However, neurotensin (8–13) was one order of magnitude more potent than native neurotensin (pEC_{50} values were 11.8±0.2 vs. 10.8±0.4 M, respectively, $P=0.04$). In contrast to the potent effects of the C-terminal fragment of neurotensin, the N-terminal fragment, neurotensin (1–6), assessed at concentrations 10^{-9}, 10^{-8}, and 10^{-7} M, was found to be ineffective as a positive inotrope (Fig. 4B). Moreover, [D-Trp^{11}]-neurotensin, a neurotensin analogue, where Tyr^{11} in the C-terminal portion is substituted by D-Trp^{11}, was only able to produce contractile effects between 19±5% and 34±8% within the concentration range of 10^{-15} to 10^{-7} M (Fig. 4C). At the highest concentration examined (10^{-7} M), the response was markedly reduced in comparison to the maximal response elicited by native neurotensin (34±8% vs. 78±5%, respectively, $P=0.002$). A uniform concentration–response relationship ($P=0.78$, repeated measures ANOVA) prevented a pEC_{50} value for [D-Trp^{11}]-neurotensin from being determined.

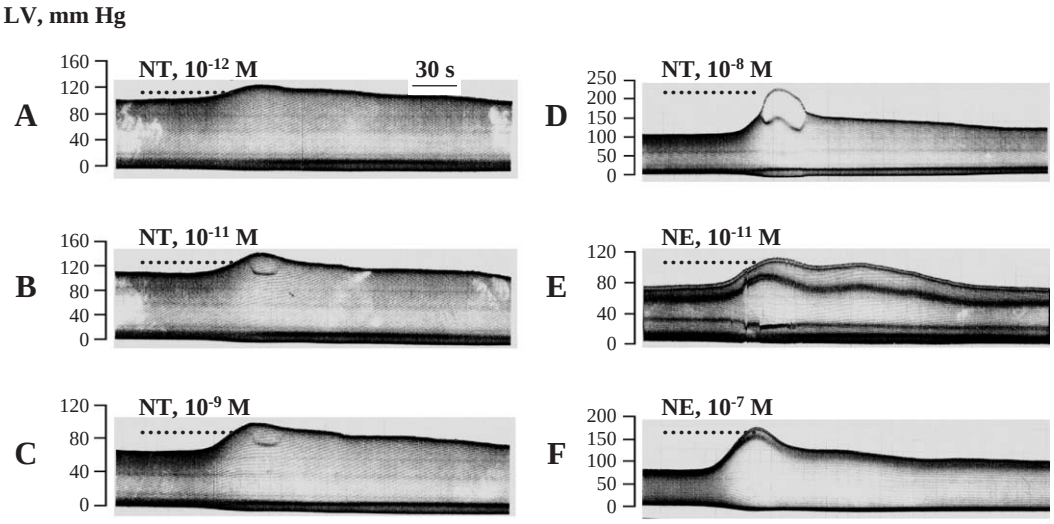

Fig. 1. Representative traces of change in left ventricular developed pressure in response to neurotensin (panels A–D) and norepinephrine (panels E–F) infusions in isolated rat left ventricles. LV, left ventricular; NT, neurotensin; NE, norepinephrine. Dots indicate the duration of infusions.

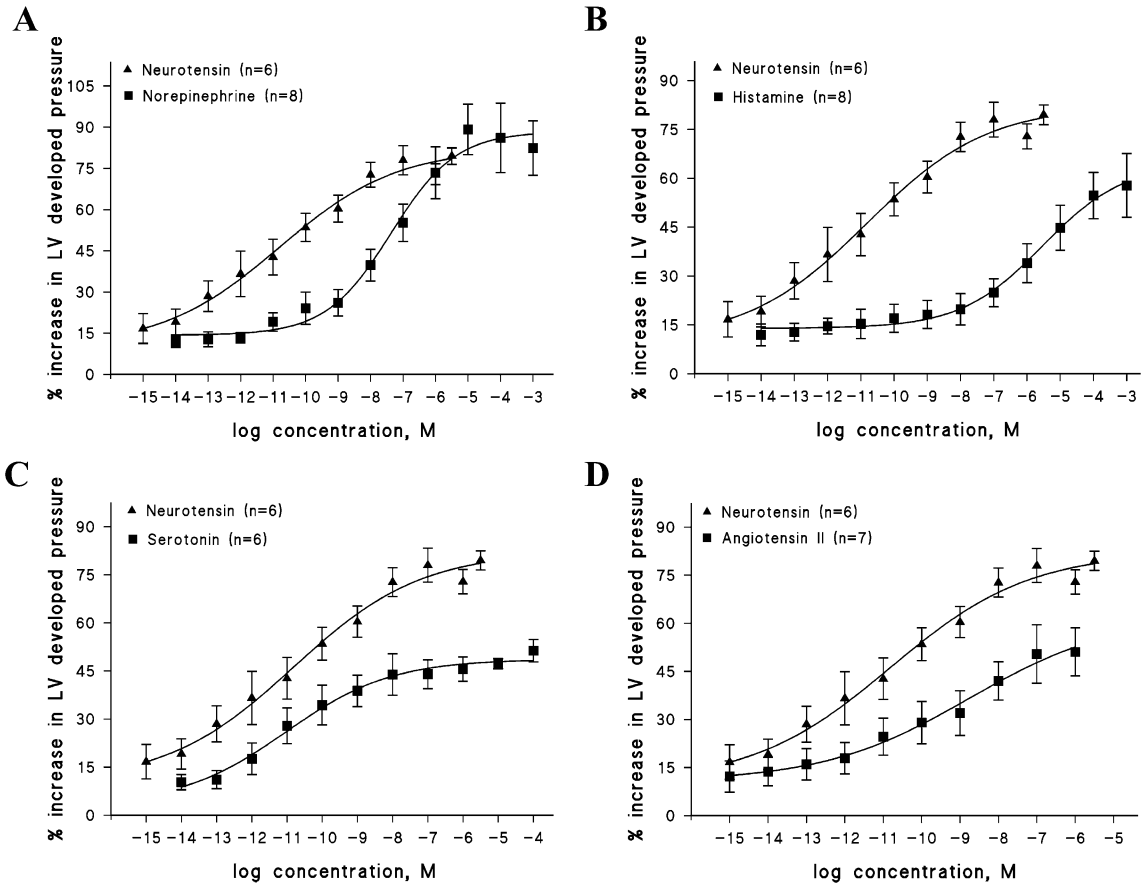

Fig. 2. A comparison of inotropic responses elicited by neurotensin with that of other endogenous inotropes in the isolated rat left ventricle. Concentration (log *M*)–response (% change in left ventricular (LV) developed pressure) curves are provided comparing effects of neurotensin and either norepinephrine (panel A), histamine (panel B), serotonin (panel C), or angiotensin II (panel D). Statistical comparisons of potency and efficacy are provided in Fig. 3.

3.4. Impact of receptor blockade on neurotensin-induced contractile responses

Neurotensin-induced contractile responses were dose-dependently inhibited by SR 48692, a specific neurotensin receptor antagonist (Fig. 5). At a concentration of 10^{-8} M, SR 48692 inhibited neurotensin-induced inotropic effects by 40% (76±15% before vs. 35±10% after antagonist, $P<0.05$) and with a higher concentration of the antagonist (10^{-7} M) the neurotensin effect was completely abolished (72±12% before vs. 7±3% after antagonist, $P<0.001$).

The contractile effects elicited by neurotensin at a dose of 10^{-8} M were unchanged by cimetidine (histamine H_2-receptor antagonist), methysergide (a non-selective serotonin receptor antagonist), or losartan, (an AT_1-receptor antagonist) (Fig. 5). However, neurotensin-induced inotropic responses were almost completely abolished following blockade of myocardial β-adrenoceptors (Fig. 5). Indeed, prior infusion of propranolol, a non-selective β-adrenoceptor antagonist, reduced neurotensin-induced inotropic responses from 75±12% to 12±4% ($P<0.01$). The abrupt inhibition of neurotensin-induced contractile effects was reproduced by atenolol, a selective β_1-adrenoceptor antagonist (before=76±17% vs. after=13±5%, $P<0.01$) (Fig. 5).

3.5. Neurotensin-mediated effects on myocardial norepinephrine release

Neurotensin (10^{-8} M) increased coronary effluent norepinephrine concentrations by 75% (Fig. 6A). Moreover, a strong positive correlation was found between neurotensin-induced increases in norepinephrine release and contractile responses (Fig. 6B).

4. Discussion

In the present study we provide the first evidence to indicate that neurotensin, a neuropeptide found in and released from ventricular tissue, elicits a marked, dose-dependent positive inotropic response in isolated, perfused rat left ventricles. The striking inotropic actions of this neuropeptide are underscored when considering that its contractile efficacy is at least equivalent to the reference endogenous inotropic agents norepinephrine and histamine, and that its potency is superior to that of these agents and angiotensin II, but at least equivalent to that of another recognized endogenous inotrope, serotonin. Neurotensin-induced inotropic responses were largely attributed to the C-

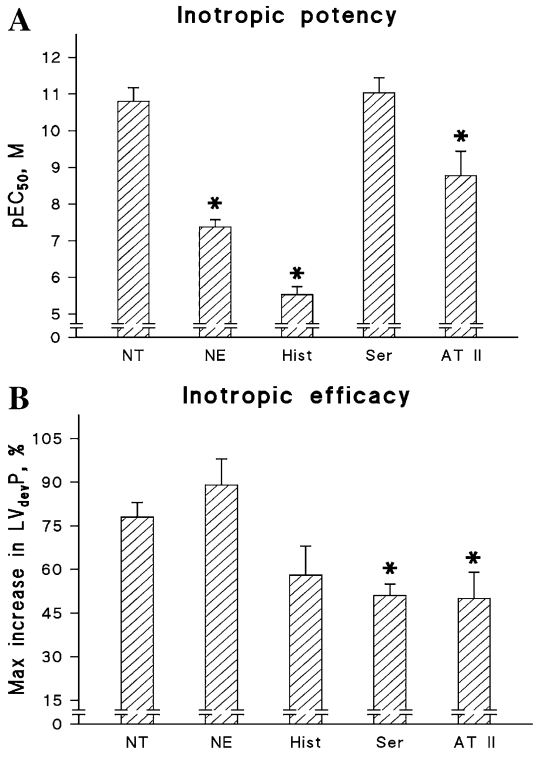

Fig. 3. A comparison of the contractile potency (pEC_{50}, panel A) and efficacy (maximal increase in left ventricular developed pressure, panel B) of neurotensin with that of other endogenous inotropes in the isolated rat left ventricle. $LV_{dev}P$, left ventricular developed pressure; Max, maximal; NT, neurotensin ($n=6$); NE, norepinephrine ($n=8$); Hist, histamine ($n=8$); Ser, serotonin ($n=6$); AT II, angiotensin II ($n=7$); pEC_{50}, $-\log_{10} EC_{50}$. * $P<0.05$ vs. corresponding value for NT.

terminal portion of this molecule, which is known to be responsible for activation of neurotensin receptors (Kitabgi et al., 1977; Sadoul et al., 1984; Gilbert et al., 1986). This finding is also supported by the ability of a selective neurotensin receptor antagonist, SR 48692, to inhibit, in a concentration-dependent manner, neurotensin-induced contractile effects. We also provide data to support a notion that neurotensin-induced inotropic responses are mediated through stimulation of myocardial norepinephrine release, as evidenced by (i) the ability of neurotensin to markedly elevate coronary effluent norepinephrine concentrations, (ii) a strong positive relationship between neurotensin-induced myocardial norepinephrine release and contractile responses, and (iii) the ability of both non-selective β-adrenoceptor and selective β_1-adrenoceptor antagonists to abolish neurotensin-induced inotropic effects.

Although a positive inotropic influence of neurotensin in isolated guinea-pig and rat atria has previously been demonstrated (Quirion et al., 1978, 1980b; Stene-Larsen and Helle, 1979), prior studies have failed to show similar effects on ventricular systolic function (Rosell et al., 1976; Rigel et al., 1989; Ertl et al., 1993). The present study provides the first clear evidence for a marked inotropic effect of neurotensin in the intact left ventricle. The apparent discrepancy between our findings and those of other authors are likely to be ascribed to differences in the preparations utilized. Although neurotensin is unable to alter left ventricular systolic segmental shortening following an intracoronary infusion in dogs (Ertl et al., 1993), or systolic function in unpaced, isolated working rabbit heart

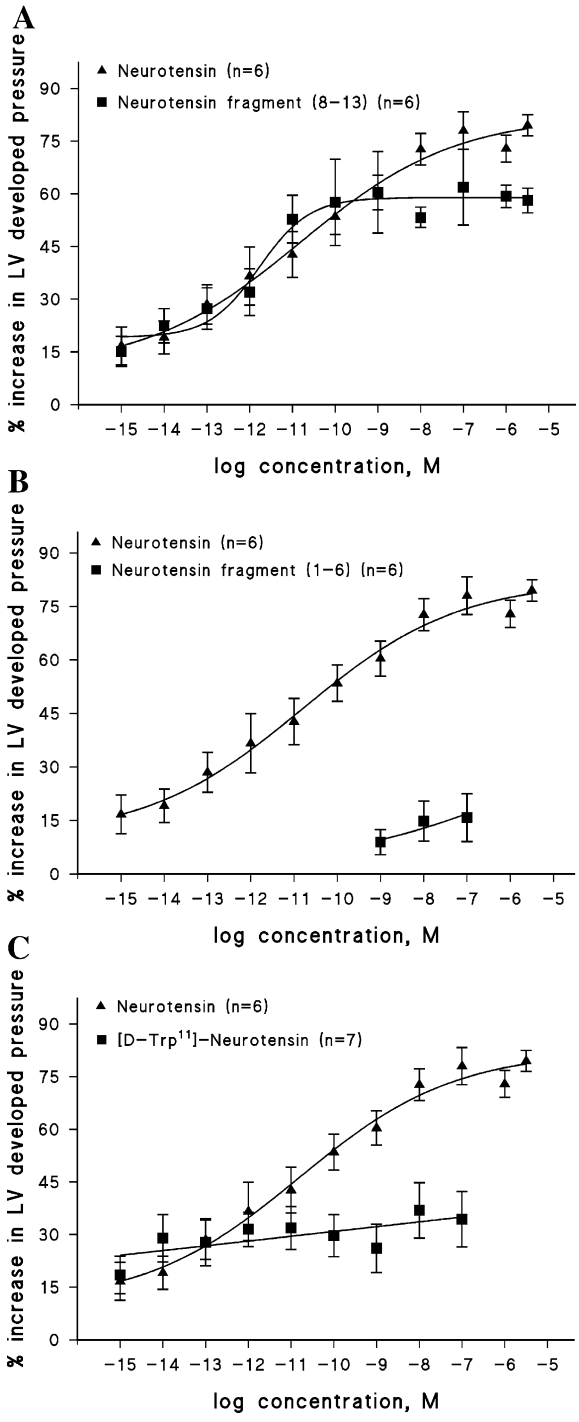

Fig. 4. A comparison of concentration (log *M*)–response (% change in left ventricular developed pressure) curves elicited by native neurotensin and its C-terminal fragment (neurotensin [8–13]) (panel A), N-terminal fragment (neurotensin [1–6]) (panel B), and analogue [D-Trp^{11}]-neurotensin (panel C) in the isolated rat left ventricle. LV, left ventricular. See text for explanation.

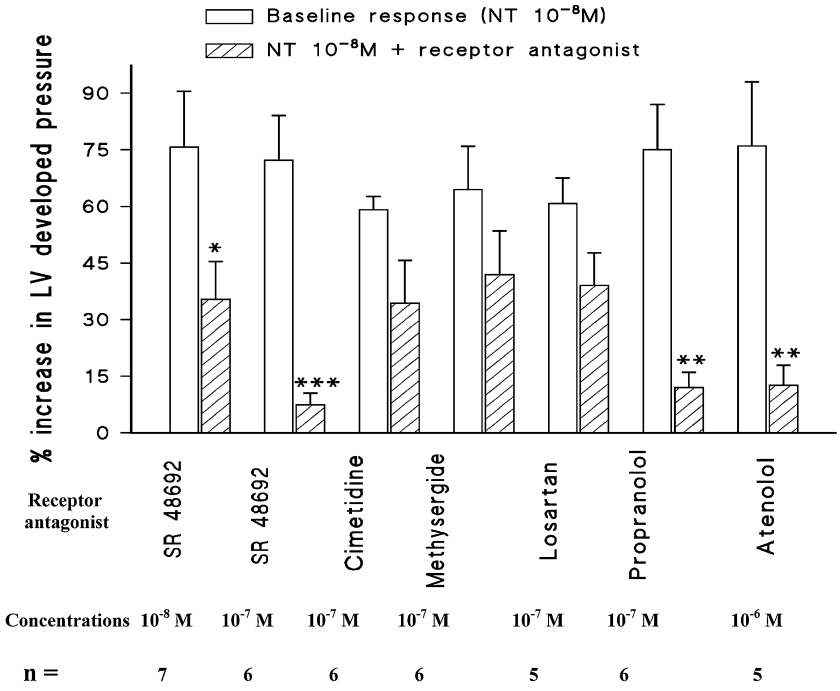

Fig. 5. Impact of receptor blockade on neurotensin (10^{-8} M)-induced contractile responses in the rat left ventricle. Neurotensin (NT), histamine H_2-, serotonin, angiotensin II receptor, non-selective β-adrenoceptor, and β_1-selective adrenoceptor blockade was achieved with SR 48692, cimetidine, methysergide, losartan, propranolol and atenolol, respectively. LV, left ventricular. * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ vs. baseline response to NT.

preparations (Rosell et al., 1976), its effect on the cardiac pacemaker (Quirion et al., 1978; Osadchii et al., 2001), arterial blood pressure (Carraway and Leeman, 1973) and coronary vascular tone (Quirion et al., 1980a; Ertl et al., 1993) were not appropriately accounted for in these studies (Rosell et al., 1976; Ertl et al., 1993). Thus changes in cardiac loading conditions, heart rate and coronary blood flow could have masked the inotropic influence of neurotensin. In this regard, in the present study we assessed neurotensin effects on left ventricular systolic function using a constant flow, paced and isovolumic isolated heart preparation. The lack of impact of neurotensin on isolated ventricular trabeculae muscle (Rigel et al., 1989) could be ascribed to a poverty of sympathetic innervation in this tissue.

The pEC_{50} value for neurotensin-induced contractile actions noted in the present study (10.8±0.4 M) is of particular importance with respect to the relevance of its effects. First, neurotensin was found in nanogram quantities in the rat left ventricle, a finding consistent with others (Goedert et al., 1984; Onuoha et al., 1999a,b). Second, all three subtypes of neurotensin receptors are expressed in the rat heart (Tanaka et al., 1990; Chalon et al., 1996; Lin et al., 1997) and the dissociation constant of the neurotensin receptor has been determined to be 10^{-9}–10^{-10} M (Kitabgi et al., 1977; Sadoul et al., 1984; Gilbert et al., 1986; Mills et al., 1988). Hence, the potency of neurotensin as a ventricular inotrope shown in the present study is very close to low concentrations expected to occur under physiological conditions and the capacity of neurotensin to bind to its receptor. Moreover, the presence of neurotensin in the coronary effluent suggests that this peptide is not only contained in, but also tonically released from the heart at concentrations comparable with those previously found in rat plasma (Rokaeus et al., 1982; Theodorsson-Norheim et al., 1985). To our knowledge, this is the first demonstration of local neurotensin release from cardiac tissue.

In the present study the C-terminal fragment of neurotensin, H-Arg^8-Arg^9-Pro^{10}-Tyr^{11}-Ile^{12}-Leu^{13}-OH, was noted to be the active component of native neurotensin at eliciting contractile responses. Indeed, the N-terminal fragment, neurotensin (1–6), was almost devoid of inotropic activity, whereas the C-terminal hexapeptide fragment, neurotensin (8–13), elicited responses very similar to those of native neurotensin. A modest difference in the magnitude of inotropic responses observed at very high concentrations (10^{-6} and $10^{-5.5}$ M) of these peptides might be attributed to an additional contribution from a non-specific, receptor-independent action of native neurotensin requiring a long hydrophobic main chain of amino acids and a positively charged Lys^6 (Mousli et al., 1990). On the other hand, an increased inotropic potency of the C-terminal hexapeptide fragment, neurotensin (8–13), as compared to native neurotensin shown in the present study is not surprising considering that the C-terminal hexapeptide has a several fold higher affinity than native neurotensin for the neurotensin receptor (Kitabgi et al., 1977) and is more potent than native neurotensin at promoting intracellular cGMP accumulation and inositoltrisphosphate formation

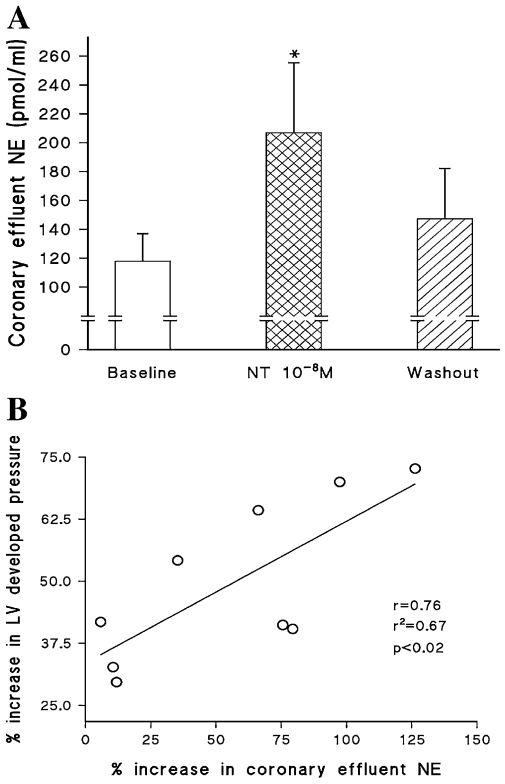

Fig. 6. Neurotensin (10^{-8} M)-induced increases in coronary effluent norepinephrine concentrations (panel A), and correlation between neurotensin-induced myocardial norepinephrine release and contractile responses (% increase in left ventricular developed pressure) (panel B). LV, left ventricular; NT, neurotensin; NE, norepinephrine. * $P=0.03$, $n=9$, vs. baseline NE concentration.

(Gilbert et al., 1986). Taken together, these findings are consistent with the notion that the C-terminal part of neurotensin is necessary for inducing the cardiovascular effects of this peptide. Indeed, the integrity of the C-terminal part of the neurotensin molecule has been shown to be critical in mediating a positive inotropic influence in isolated guinea-pig atria (Quirion et al., 1980b), coronary vasoconstriction in isolated rat hearts (Quirion et al., 1980a), and contractile effects in isolated rat portal vein preparations (Rioux et al., 1980). It has been suggested that Arg^{9} and Pro^{10} contribute to neurotensin affinity for its receptor, whereas Tyr^{11} is more important for full activation of the receptor (Quirion et al., 1980b). Indeed, in the present study the neurotensin analogue, [D-Trp^{11}]-neurotensin, was found to be much less effective at eliciting positive inotropic responses in comparison to that of native neurotensin.

A pivotal role of neurotensin receptor activation in mediating contractile responses elicited by this neuropeptide is further supported by the ability of SR 48692 to dose-dependently inhibit neurotensin-induced inotropic effects. SR 48692 is a specific nonpeptide neurotensin receptor antagonist that competitively inhibits [^{125}I]-labeled neurotensin binding to high-affinity receptors in the brain (Gully et al., 1993). Moreover, SR 48692 abolishes the tachycardia and positive inotropic responses elicited by neurotensin in isolated spontaneously beating atria (Nisato et al., 1994).

A shallow neurotensin-induced dose–contractile response curve (Hill slope less than a unit) is consistent with the existence of multiple receptor subtypes contributing to the contractile effects of this neuropeptide. Indeed, both high-affinity (NTS1) and low-affinity (levocabastine-sensitive, nts2) neurotensin binding sites have been demonstrated in the rat myocardium (Tanaka et al., 1990; Chalon et al., 1996). Moreover, a recently cloned neurotensin receptor, nts3 (sortilin), has been found to be expressed in heart muscle (Lin et al., 1997). The relative contribution of these receptor subtypes in mediating neurotensin-induced contractile responses is presently unknown. The effects of the neurotensin receptor antagonist, SR 48692 as described by us, does not shed light on the specific receptor involved as SR 48692, at the concentrations employed in the present study (10^{-8}–10^{-7} M), does not selectively inhibit neurotensin receptor subtypes (Gully et al., 1993).

The neurotensin receptor is coupled to phospholipase C, which induces inositoltrisphosphate formation and subsequently promotes calcium release from intracellular stores (Gilbert et al., 1986). These changes could contribute to neurotensin-induced contractile effects through direct post-synaptic actions, or through presynaptic release of other endogenous substances with inotropic properties. Indeed, previous studies indicate that neurotensin stimulates histamine release in the isolated rat heart (Rioux et al., 1984), promotes combined histamine and serotonin release in the rat perfused hindquarter (Kerouac et al., 1984), and evokes endogenous norepinephrine release from rat hypothalamic slices (Okuma and Osumi, 1982). However, in the present study neither histamine, serotonin, nor angiotensin II receptor blockade modified neurotensin-induced contractile effects. Alternatively, neurotensin-induced inotropic responses were abolished by both non-selective (propranolol) and β_1-selective (atenolol) antagonists of myocardial β-adrenoceptors. Moreover, neurotensin increased endogenous norepinephrine release and a positive correlation between neurotensin-induced myocardial norepinephrine release and contractile responses was noted. These data support a neuromodulatory action of neurotensin on sympathetic presynaptic terminals in rat left ventricle.

In the present study, neurotensin was found to be about three times more potent than norepinephrine at eliciting contractile responses. This difference could be explained by the ability of neurotensin to stimulate presynaptic norepinephrine release at very low concentrations of neurotensin. Although the magnitude of neurotensin-induced norepinephrine release was fairly substantial (+75%), it does not account for the large differences in contractile potency between neurotensin and norepinephrine. This apparent discrepancy is explained by the fact that neurotensin-induced increases in norepinephrine release most likely produces a markedly greater norepinephrine concentration in the synaptic cleft as compared to the coronary effluent. This

may occur as about 80–90% of released norepinephrine is taken up again in the presynaptic terminal (Esler et al., 1990) and myocardial capillaries provide a substantial barrier to norepinephrine diffusion from the interstitial space to the coronary circulation (Cousineau et al., 1986).

In conclusion, the present study indicates that neurotensin, through modulation of myocardial norepinephrine release, exerts a potent positive inotropic influence on the ventricular myocardium. Further work is required to determine whether these findings translate into a new target for modifying myocardial contractility.

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