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Neurotensin causes tyrosine phosphorylation of focal adhesion kinase in lung cancer cells

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Abstract

The effects of neurotensin on focal adhesion kinase were investigated using lung cancer cells. Neurotensin bound with high affinity to large cell carcinoma cell line NCI-H1299 as did neurotensin-(8–13), but not neurotensin-(1–7) or levocabastine. Addition of 100 nM neurotensin to NCI-H1299 cells caused transient tyrosine phosphorylation of focal adhesion kinase which was maximal after 1–2.5 min. Also, neurotensin-(8–13), but not neurotensin-(1–8) or levocabastine, caused tyrosine phosphorylation of focal adhesion kinase after addition to NCI-H1299 cells. Focal adhesion kinase tyrosine phosphorylation caused by neurotensin was inhibited by the nonpeptide neurotensin receptor antagonist (2-(1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1*H*-pyrazole-3-carbonyl)amino)-adamantane-2-carboxylic acid) (SR48692). SR48692 inhibited the clonal growth of NCI-H1299 cells, whereas neurotensin-stimulated proliferation and levocabastine had no effect. These results indicate that lung cancer cells have functional neurotensin receptors which regulate focal adhesion kinase tyrosine phosphorylation. It remains to be determined if neurotensin receptors and focal adhesion kinase plays a role in lung cancer cellular adhesion and migration. © 2002 Published by Elsevier Science B.V.

Keywords: Lung cancer; Focal adhesion kinase; Neurotensin; SR48692; Proliferation

1. Introduction

Neurotensin, a tridecapeptide, is biologically active in the central nervous system, where it binds with high affinity to neuronal receptors (Carraway and Leeman, 1973; Kitabgi et al., 1977). Exogenous administration of neurotensin into the central nervous system causes analgesia, hypotension and hypothermia (Clineschmidt et al., 1979; Nemeroff et al., 1979; Rioux et al., 1980). Immunoreactive neurotensin has been identified in extracts of neuroendocrine cells including small cell lung cancer and medullary thyroid carcinoma (Wood et al., 1981; Moody et al., 1985; Zeytinoglu et al., 1995). Neurotensin is secreted from prostate cancer cells and tumors resulting in high patient plasma neurotensin levels (Sehgal et al., 1994; Kapuscinski et al., 1990). After receptor activation, neurotensin is degraded by the enzyme endopeptidase 24.11 (Cohen et al., 1996; Moody et al., 1998).

Neurotensin alters the growth of cancer cells and increases small cell lung cancer colony formation 2.5-fold (Davis et al., 1989, 1991). Also, neurotensin elevates cytosolic Ca^{2+} in small cell lung cancer cells (Staley et al., 1989). Two types of neurotensin receptors have been identified, NTS1 (neurotensin$_1$) and NTS2 (neurotensin$_2$) receptors which are G-protein coupled receptors containing 418 and 410 amino acids, respectively (Betancur et al., 1998). NTS1 receptors bind neurotensin with high affinity and are antagonized by nonpeptide antagonists such as 2-(1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1*H*-pyrazole-3-carbonyl)amino)-adamantane-2-carboxylic acid (SR48692) or 2-[[5-(2,6-dimethoxyphenyl)-1-(4-*N*-(3-dimethylaminopropyl)-*N*-methylcarbamoyl)-2-isopropylphenyl)-1*H*-pyrazole3-carbonyl]amino]adamantane-2-carboxylic acid (SR142948A) (Gully et al., 1993, 1997). NTS1 receptors activate guanine nucleotide-binding subunits (Gq) causing phosphatidylinositol turnover. The inositol-1,4,5-trisphosphate released causes Ca^{2+} release from intracellular organelles, whereas the diacylglycerol released activates protein kinase C. Protein kinase C may phosphorylate mitogen-activated protein kinase leading to mitogen-activated protein

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kinase phosphorylation. Phosphorylated mitogen-activated protein kinase enters the nucleus and activates substrates such as Elk-1. Elk-1, in turn, increases expression of early oncogenes such as c-*fos* and c-*jun* (Whitmarsh and Davies, 1996). c-*fos* and c-*jun* may form heterodimers in increase expression of growth factor genes. In contrast, NTS2 receptors bind the histamine receptor antagonist levocabastine as well as neurotensin with moderate affinity (Yamada et al., 1998). Also, the NTS3 receptor, which is not coupled to G proteins, has been cloned (Mazella et al., 1998).

Neuropeptides stimulate the tyrosine phosphorylation of focal adhesion kinase, a novel tyrosine kinase (Rozengurt, 1991; Zachary et al., 1987). The increases in focal adhesion kinase and paxillin tyrosine phosphorylation caused by bombesin were accompanied by reorganization of the actin cytoskeleton and by the assembly of focal adhesion plaques where both focal adhesion kinase and paxillin are localized (Schaller et al., 1992; Hanks et al., 1992). The ability of various growth factors to cause tyrosine phosphorylation of focal adhesion kinase is dependent on the integrity of the actin cytoskeleton (Leeblundberg et al., 1994; Rankin and Rozengurt, 1994; Taniguchi et al., 1996; Zachary et al., 1993). Focal adhesion kinase is involved in cancer cell adhesion, spreading, migration, proliferation and survival (Jones et al., 2000).

In the present study, the effects of neurotensin on tyrosine phosphorylation of focal adhesion kinase were investigated in lung cancer cells. Our results indicate that neurotensin causes tyrosine phosphorylation of focal adhesion kinase and the increase in tyrosine phosphorylation caused by neurotensin was inhibited by SR48692. Also, neurotensin stimulated the clonal growth of NCI-H1299 cells, whereas SR48692 inhibited proliferation.

2. Materials and methods

2.1. Cell culture

Non-small cell lung cancer cell lines NCI-H157 and NCI-H1299 were cultured in RMPI-1640 (Life Technologies, Rockville, MD) containing 10% heat-inactivated fetal bovine serum (Carney et al., 1985). The cells, which are adherent, were split 1/20 weekly after treatment with trypsin/ethylenediaminotetraacetic acid. The cells were mycoplasma free and cultured at 37 °C in 5% CO_2/95% air.

2.2. Receptor binding

The ability of neurotensin ligands to bind with high affinity to non-small cell lung cancer cells was investigated. NCI-H1299 cells were plated in 24-well plates. When a monolayer of cells formed, they were washed three times in SIT medium (RPMI-1640 containing 3×10^{-8} M sodium selenite, 5 μg/ml bovine insulin and 10 μg/ml transferrin (Sigma, St. Louis, MO)). Cells were placed in receptor-binding medium (RPMI-1640 containing 0.1% bovine serum albumin and 100 μg/ml bacitracin in the presence or absence of competitor; SR48692, which has limited solubility in water, was dissolved in DMSO and a 10,000 μM stock was diluted for use in vitro. ^{125}I-Neurotensin (2200 Ci/mmol, New England Nuclear, Boston, MA) was added for 60 min at 25 °C. After equilibrium binding was attained (Allen et al., 1988), NCI-H1299 cells were washed three times in receptor-binding medium to remove free radiolabeled peptide and the cells containing bound peptide dissolved in 0.2 N NaOH. The samples were placed in a counting tube and counted in a LKB gamma counter. Nonspecific binding was approximately 20% of the total binding.

2.3. Western blot

The ability of neurotensin-like peptides (Phoenix Pharmaceuticals, Belmont, CA) to stimulate tyrosine phosphorylation of focal adhesion kinase was investigated by Western blotting (Garcia et al., 1997). NCI-H1299 cells were maintained in SIT containing 0.5% fetal bovine serum overnight. Three hours before treatment, cells were preincubated in ST media (RPMI-1640 containing 3×10^{-8} M Na_2SeO_3 and 10 μg/ml transferrin (Sigma)). Cells were treated with different neurotensin ligands for 2.5 min, washed with saline and lysed with 1 ml lysis buffer containing 50 mM Tris–HCl (pH 7.5), 150 mM sodium chloride, 1% (w/v) Triton X-100, 1% (w/v) deoxycholate, 1% (w/v) NaN_3, 1 mM ethyleneglycol bis(β-aminoethylether)-*N*,*N*,*N*,*N*′ -tetraacetic acid, 0.4 mM ethylene diamino tetraacetic acid, 2.5 μg/ml aprotinin, 2.5 μg/ml leupeptin, 1 mM phenylmethylsulfonylfluoride and 0.2 mM sodium vanadate (Sigma). The lysate was sonicated for 5 s at 4 °C and centrifuged at $10{,}000 \times g$ for 15 min. Protein concentration was measured by Bio-Rad (Richmond, CA) protein assay reagent, and the volume was adjusted such that 1 ml aliquot of cell lysates contained the same amount of protein (150 μg/ml). The lysates were incubated with 4 μg of anti-phosphotyrosine monoclonal antibody (PY20), 4 μg of rabbit anti-mouse IgG and 30 μl of Protein A-agarose overnight at 4 °C. Immunoprecipitates were fractionated on sodiumdodecylsulfate/polyacrylamide gel electrophoresis using 10% polyacrylamide gels (Novex, Carlsbad, CA), and proteins with molecular masses higher than 60 kDa were transferred to nitrocellulose membranes. Membranes were blocked overnight at 4 °C using blotto [5% nonfat dried milk in a solution containing 50 mM Tris–HCl, pH 8.0, 2 mM calcium chloride, 80 mM sodium chloride and 0.05% (v/v) Tween 20. Then the membranes were incubated for 2 h at 25 °C with 0.25 μg/ml of a anti-focal adhesion kinase monoclonal antibody (Upstate Biotechnologies, Lake Placid, NY). The membranes were washed twice for 10 min each with blotto and incubated for 40 min at 25 °C with anti-mouse IgG-horseradish peroxidase conjugate. The membrane was washed for 10 min with blotto, followed by two 10-min treatments with washing

solution [50 mM Tris–HCl, pH 8.0, 2 mM calcium chloride, 80 mM sodium chloride and 0.05% (v/v) Tween 20, incubated with enhanced chemiluminescence detection reagent for 5 min and exposed to Hyper film ECL (Amersham, Arlington Heights, IL). The density of bands on the film was measured using a scanning densitometer.

2.4. Clonogenic assay

For the proliferation experiments, growth studies were performed in vitro using NCI-H1299 cells and an agarose cloning assay system (Mahmoud et al., 1991). The base layer consisted of 3 ml of 0.5% agarose (FMC) in SIT medium containing 5% fetal calf serum in 6-well plates. The top layer consisted of 3 ml of SIT medium in 0.3% agarose, neurotensin or SR48692 and 5×10^4 single viable cells. For each drug concentration, triplicate wells were plated. After 2 weeks, 1 ml of 0.1% *p*-iodonitrotetrazolium violet was added, and after 16 h at 37 °C, the plates were screened for colony formation; the number of colonies larger than 50 μm in diameter were counted using an Omnicon image analysis system.

3. Results

3.1. Neurotensin and SR48692 bind with high affinity to lung cancer cells

The ability of neurotensin-like ligands to bind to NCI-H1299 cells was investigated. Table 1 shows that neurotensin inhibited specific ^{125}I-neurotensin binding to NCI-H1299 cells with high affinity. The IC_{50} values for neurotensin, SR48692 and neurotensin-(8–13) were 3, 50 and 10 nM, whereas neurotensin-(1–8) and levocabastine bound with low affinity (IC_{50} value>1000 nM). The results indicate that the C-terminal of neurotensin, but not levocabastine, binds with high affinity to NCI-H1299 cells. Similar results were obtained using NCI-H157 cells (data not shown).

3.2. Neurotensin causes focal adhesion kinase tyrosine phosphorylation

The time course for neurotensin to tyrosine phosphorylate focal adhesion kinase was investigated. Fig. 1 (top)

Table 1
Binding of neurotensin (NT)-like ligands

Addition	IC_{50} (nM)
NT	3 ± 1
NT-(8–13)	10 ± 2
NT-(1–7)	>1000
Levocabastine	>1000
SR48692	50 ± 5

The IC_{50} to inhibit specific ^{125}I-NT binding to NCI-H1299 cells is indicated. The mean value ± S.D. of four determinations each repeated in quadruplicate is indicated.

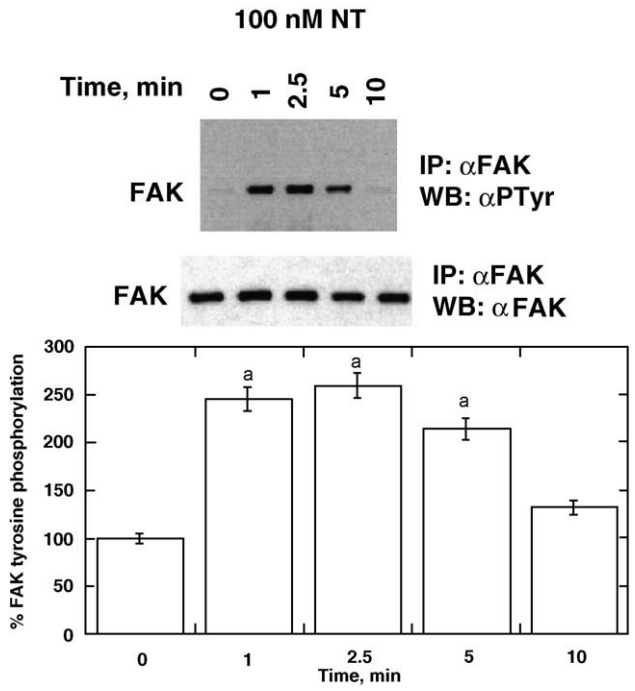

Fig. 1. Time course of focal adhesion kinase (FAK) tyrosine phosphorylation. Top: The ability of 100 nM neurotensin (NT) to tyrosine phosphorylate FAK was investigated as a function of time using an anti (α) FAK antibody for immunoprecipitation (IP) and α phosphotyrosine (PTyr) antibody for the Western blot (WB). Middle: The amount of FAK loaded onto the gel was determined using α FAK antibody for IP and α FAK antibody for the WB. Bottom: This mean percent increase ± S.D. of four experiments is indicated; $^a p < 0.05$ by ANOVA.

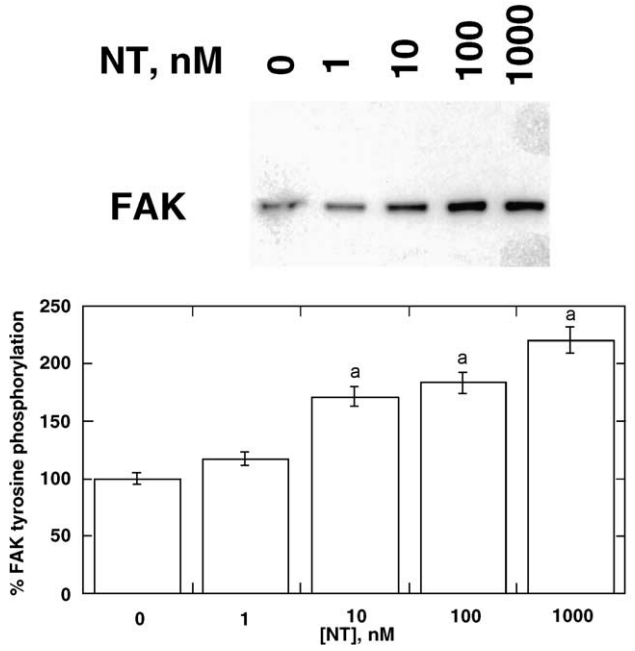

Fig. 2. Dose–response curve. Top: NT was added at different concentrations to NCI-H1299 cells and the ability to tyrosine phosphorylate FAK determined after 2.5 min. Bottom: This mean percent increase ± S.D. of three experiments is indicated; $^a p < 0.05$ by ANOVA.

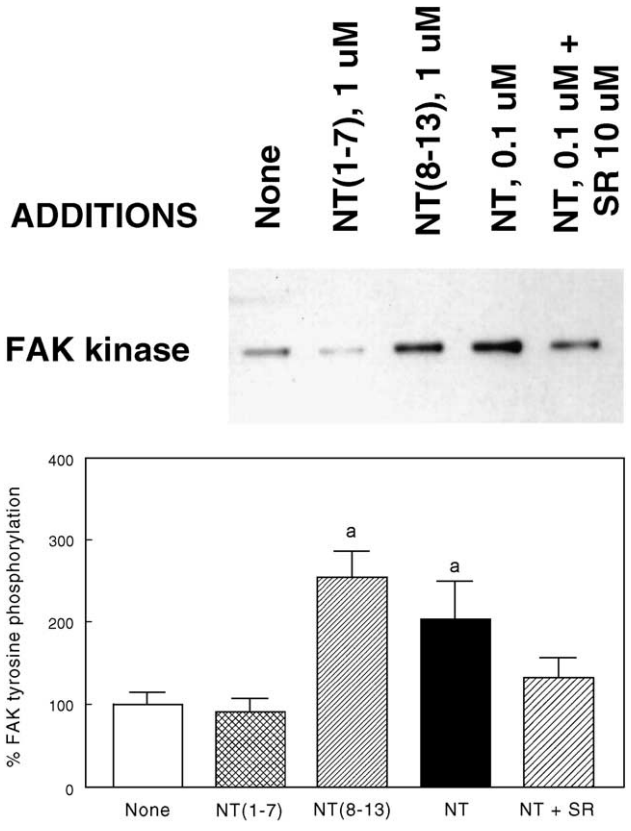

Fig. 3. Specificity of tyrosine phosphorylation. Top: The ability of 1 μM NT-(1–7), 1 μM NT-(8–13), 0.1 μM NT and 0.1 μM NT plus 10 μM SR48692 (SR) to tyrosine phosphorylate FAK was determined 2.5 min after addition to NCI-H1299 cells. Bottom: The mean percent increase ± S.D. of three experiments is indicated; $^{a}p < 0.05$ by ANOVA.

shows that after addition of 100 nM neurotensin to NCI-H1299 cells, tyrosine phosphorylation was maximal after 1 to 2.5 min using anti-focal adhesion kinase antibody to immunoprecipitate proteins and anti-phosphotyrosine antibody in the Western blot. Tyrosine phosphorylation caused by neurotensin started to decline at 5 min and returned to basal levels after 10 min. Fig. 1 (bottom) shows that 100 nM neurotensin increased significantly focal adhesion kinase tyrosine phosphorylation 2–2.5-fold after 1, 2.5 and 5 min. These results indicate that neurotensin transiently increases focal adhesion kinase tyrosine phosphorylation in NCI-H1299 cells, and similar results were obtained for NCI-H157 cells (data not shown). As a control, equal amounts of FAK protein were loaded onto the gel using anti-focal ahesion kinase to immunoprecipitate proteins and anti-focal adhesion kinase antibody in the Western blot (Fig. 1, middle). In all subsequent experiments, equal amounts of focal adhesion kinase protein were loaded onto the gels (data not shown).

The dose–response curve for neurotensin to cause tyrosine phosphorylation of focal adhesion kinase was determined. Fig. 2 (top) shows that 2.5 min after addition of 1 nM neurotensin to NCI-H1299 cells, there was little increase in focal adhesion kinase tyrosine phosphorylation. The ED_{50} was 10 nM, whereas 100 and 1000 nM neurotensin caused maximal tyrosine phosphorylation of NCI-H1299 focal adhesion kinase. Fig. 2 (bottom) shows that 10, 100 and 1000 nM neurotensin significantly increased focal adhesion kinase tyrosine phosphorylation 1.5–1.9-fold. These results indicate that neurotensin in a concentration-dependent manner caused increased tyrosine phosphorylation of focal adhesion kinase in NCI-H1299 cells.

The specificity of focal adhesion kinase tyrosine phosphorylation was investigated. Fig. 3 (top) shows that 100 nM neurotensin or 1000 nM neurotensin-(8–13), but not 1000 nM neurotensin-(1–7), caused tyrosine phosphorylation of focal adhesion kinase 2.5 min after addition to NCI-H1299 cells. Also, 10 μM SR48692 inhibited the ability of 100 nM neurotensin to cause tyrosine phosphorylation of focal adhesion kinase. Fig. 3 (bottom) shows that neurotensin and neurotensin-(8–13) significantly increased focal adhesion kinase tyrosine phosphorylation 2–2.4-fold. The increase in tyrosine phosphorylation of focal adhesion kinase caused by neurotensin was antagonized by SR48692. These results indicate that the C-terminal of neurotensin is essential to cause tyrosine phosphorylation NCI-H1299 focal adhesion kinase.

SR48692 had little effect on basal focal adhesion kinase tyrosine phosphorylation (Fig. 4, top). Addition of neurotensin (100 nM), but not levocabastine (1 μM) or SR48692 (10 μM), to NCI-H1299 cells increased significantly focal

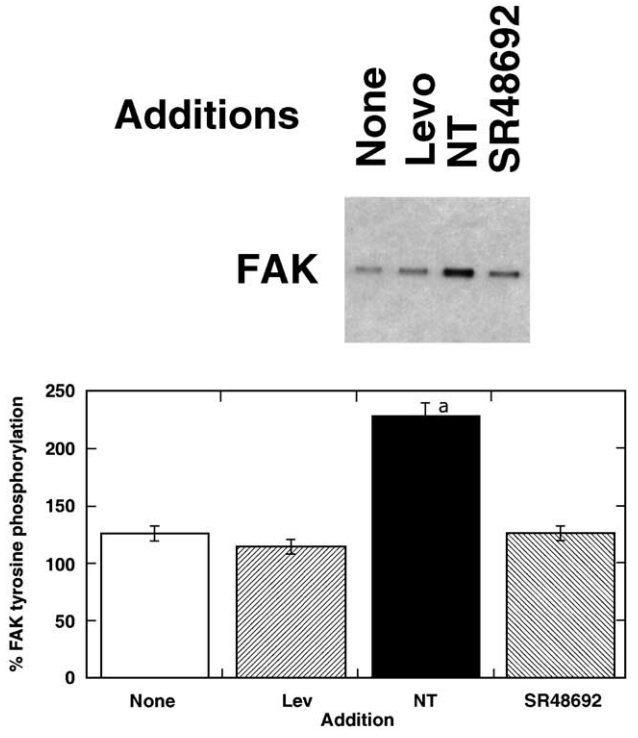

Fig. 4. Effect of NT ligands on tyrosine phosphorylation. Top: The ability of 10 μM SR48692, 1 μM levocabastine and 0.1 μM neurotensin to cause tyrosine phosphorylation of FAK was determined 2.5 min after addition to NCI-H1299 cells. Bottom: The mean value ± S.D. of three experiments is indicated; $^{a}p < 0.05$ by ANOVA.

adhesion kinase tyrosine phosphorylation 2.7-fold. These results indicate that neurotensin, but not levocabastine or SR48692, cause tyrosine phosphorylation of focal adhesion kinase.

The ability of various agents to inhibit tyrosine phosphorylation of focal adhesion kinase caused by neurotensin was investigated. Fig. 5 (top) shows that 0.3 μM cytochalasin D, which inhibits actin polymerization, reduced tyrosine phosphorylation of focal adhesion kinase caused by neurotensin. In contrast, 0.3 μM cytochalasin D had little effect on basal focal adhesion kinase tyrosine phosphorylation. Fig. 5 (bottom) shows that 0.3 μM cytochalasin D inhibited by 45% the increase in tyrosine phosphorylation of focal adhesion caused by neurotensin. In contrast, 3 μM cytochalasin D inhibited almost all of the increase in focal adhesion kinase tyrosine phosphorylation caused by neurotensin (data not shown). Fig. 6 (top) shows that the increase in focal adhesion kinase tyrosine phosphorylation caused by neurotensin was reduced by 50 μM genistein, a tyrosine kinase inhibitor, as well as 50 μM H7 (1-(5-isoquinolinesulfonyl)-2-methylpiperazine). Fig. 6 (bottom) shows that 100 nM neurotensin significantly increased focal adhesion kinase tyrosine phosphorylation 1.9-fold and that genistein as well as H7 decreased the focal adhesion kinase tyrosine phosphorylation induced by neurotensin by 58% and 76%, respectively. These results indicate that kinase inhibitors reverse the tyrosine phosphorylation of focal adhesion kinase caused by neurotensin.

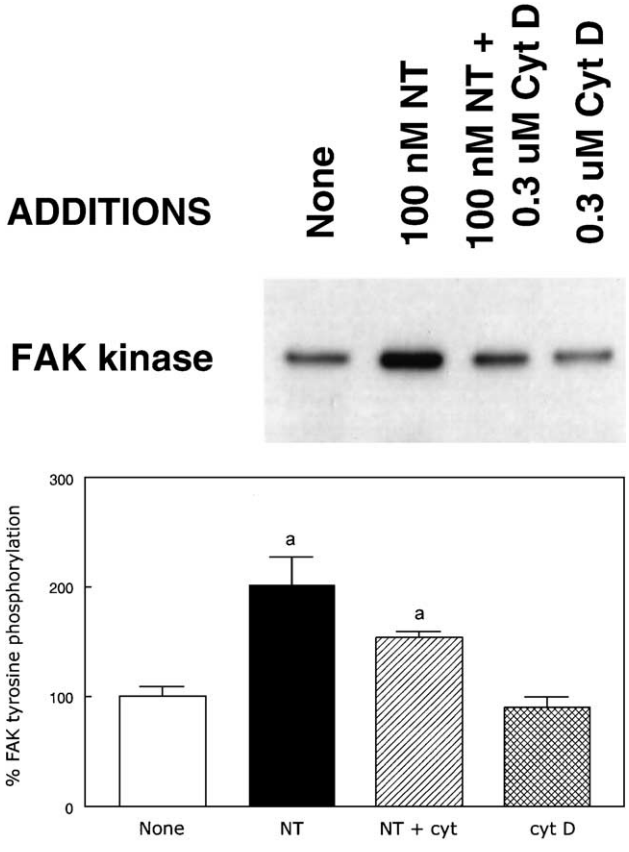

Fig. 5. Cytochalasin D and FAK tyrosine phosphorylation. Top: The ability of 0.3 μM cytochalasin D (cyt D) to inhibit FAK tyrosine phosphorylation caused by 100 nM NT was determined 2.5 min after addition to NCI-H1299 cells. Bottom: The mean percent increase ± S.D. of four experiments is indicated; $^{a}p < 0.05$ by ANOVA.

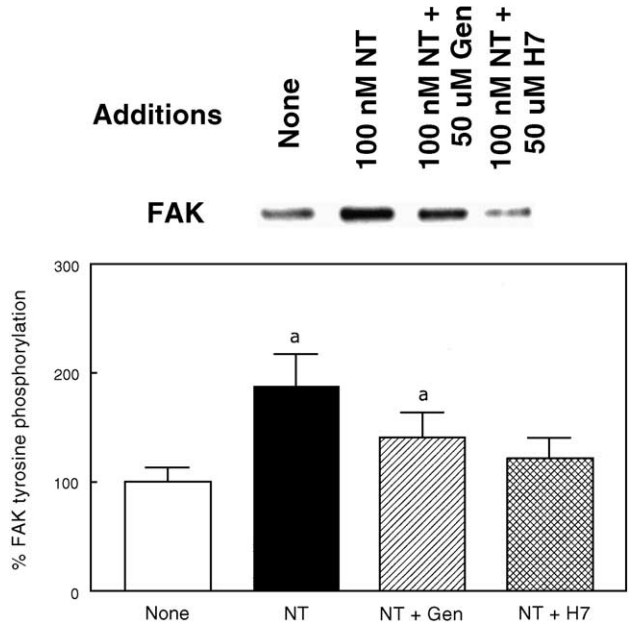

Fig. 6. FAK tyrosine phosphorylation and kinase inhibitors. Top: The ability of 50 μM genistein (Gen) and 50 μM H7 to inhibit tyrosine phosphorylation of FAK caused by 100 nM NT was determined 2.5 min after addition to NCI-H1299 cells. Bottom: The mean percent increase ± S.D. of three experiments is indicated; $^{a}p < 0.05$ by ANOVA.

3.3. Neurotensin stimulates and SR48692 inhibits lung cancer cellular proliferation

The effects of neurotensin on NCI-H1299 proliferation were investigated. Table 2 shows that the neurotensin antagonist SR48692 inhibited NCI-H209 colony proliferation by 90%. The effects of SR48692 were reversed by neurotensin, which stimulated colony formation 1.9-fold. In contrast, levocabastine had little effect on lung cancer cellular proliferation as did a solvent control (0.1% DMSO).

Table 2
Clonogenic assay

Addition	Colonies
None	114 ± 34
Levocabastine, 1 μM	121 ± 21
SR48692, 10 μM	11 ± 1[b]
NT, 10 nM	202 ± 41[a]
NT + SR48692	117 ± 11
0.1% DMSO	105 ± 8

The mean number of NCI-H1299 colonies ± S.D. of three determinations is indicated; $^{a}p < 0.05$, $^{b}p < 0.01$ by ANOVA. This experiment is representative of three others.

4. Discussion

Previously, we found that many lung cancer cell lines have immunoreactive neurotensin (Moody et al., 1985). After secretion from lung cancer cells, neurotensin binds with high affinity to neurotensin receptors and causes cytosolic Ca^{2+} elevation (Staley et al., 1989; Allen et al., 1988). Also, neurotensin increased the proliferation of lung cancer cells (Davis et al., 1989, 1991). Here, the effects of neurotensin on focal adhesion kinase tyrosine phosphorylation were investigated.

Focal adhesion kinase is tyrosine phosphorylated by a wide variety of agents, including peptides, growth factors and integrins; however, the role of neurotensin has not been investigated (Schaller et al., 1992; Zachary et al., 1993; Hanks et al., 1992). Neurotensin, similar to bombesin, binds with high affinity to small cell lung cancer cells and causes phosphatidylinositiol turnover (Allen et al., 1988). Previously, we found that bombesin-like peptides caused focal adhesion kinase tyrosine phosphorylation (Leyton et al., 2001). Here, we found that neurotensin caused focal adhesion kinase tyrosine phosphorylation in a time- and dose-dependent manner in NCI-H1299 cells. The ED_{50} for neurotensin to tyrosine phosphorylate focal adhesion kinase was 10 nM, whereas the half maximal binding of neurotensin to NCI-H1299 cells occurred at 3 nM. The C-terminal of neurotensin was essential for focal adhesion kinase tyrosine phosphorylation in that neurotensin-(8–13), but not neurotensin-(1–7), caused focal adhesion kinase tyrosine phosphorylation and bound to NCI-H1299 cells with high affinity. SR48692 inhibited high affinity ^{125}I-neurotensin binding to NCI-H1299 cells ($IC_{50} = 50$ nM) and blocked the ability of neurotensin to cause tyrosine phosphorylation of focal adhesion kinase. Because high concentrations of neurotensin (100 nM) were used to tyrosine phosphorylate focal adhesion kinase, high concentrations of SR48692 (10 μM) were required to block the increase caused by neurotensin. Preliminary data (T. Moody, unpublished) indicate that mRNA for NTS1 receptors are present in NCI-H1299 cells. Using in vitro autoradioagraphy techniques, ^{125}I-neurotensin bound with high affinity to lung cancer and Ewing's Sarcoma biopsy specimens (Reubi et al., 1999). In contrast, the NTS2 receptor agonist levocabastine had little effect on focal adhesion kinase tyrosine phosphorylation. It remains to be determined if mRNA for the NTS2 or NTS3 receptors are present in lung cancer cells (Mazella et al., 1998).

Focal adhesion kinase resides at sites where integrins cluster (Schaller et al., 1992; Zachary et al., 1993; Jones et al., 2000; Kornberg et al., 1992). Focal adhesion kinase contains a central catalytic domain that is flanked by an amino-terminal region which contains a β1-integrin-binding site (Schaller et al., 1995) and a carboxy terminal region which contains two proline-rich domains that can bind $p130^{CAS}$ or the Rho GTPase activating protein Graf. Also, the focal adhesion kinase has carboxy terminal sequences which overlap with the multi-adaptor protein paxillin (Hildebrand et al., 1996; Cary et al., 1998). Focal adhesion kinase is a tyrosine kinase which has an ATP-binding site (Akiyama et al., 1987). Upon enzyme activation, the ATP is hydrolyzed resulting in ADP and phosphorylated tyrosine. Routinely, tyrosine 397 is phosphorylated in focal adhesion kinase. Genistein in a noncompetitive inhibitor of tyrosine kinases which causes the formation of nonproductive enzyme–substrate complexes (Akiyama et al., 1987) and genistein reduced focal adhesion kinase tyrosine phosphorylation induced by neurotensin. In contrast, the kinase inhibitor H7 is a competitive inhibitor of serine, threonine kinases such as protein kinase C which competes for the ATP-binding site (Hidaka and Kabayashi, 1992). H7 reduced the focal adhesion kinase tyrosine phosphorylation caused by neurotensin using NCI-H1299 cells after 2.5 min and, previously, we found that H7 reduced the focal adhesion kinase tyrosine phosphorylation caused by bombesin (Leyton et al., 2001). Preliminary data (T. Moody, unpublished) indicate that genistein and H7 inhibit the proliferation of NCI-H1299 cells. Also, upregulation of focal adhesion kinase is associated with increased oncogene expression in prostate cancer cells (Bergan et al., 1996; Tremblay et al., 1995). Recently, we found that neurotensin increased c-*fos* mRNA in lung cancer cells (Moody et al., 2001). Focal adhesion kinase is important for integrity of the cellular cytoskeleton (Burridge et al., 1988; Leeblundberg et al., 1994). Cholecystokinin receptors regulate the formation of actin stress fibers (Taniguchi et al., 1996). Here, cytochalasin D, which disrupts the actin microfilaments, inhibited neurotensin-stimulated focal adhesion kinase tyrosine phosphorylation. The effect appears specific for actin microfilaments because colchicine, which disrupts microtubules, had little effect of the ability of neurotensin to tyrosine phosphorylate focal adhesion kinase (T. Moody, unpublished).

Previously, it was found that focal adhesion kinase antisense inhibited adhesion of human embryonal RD cells and prevented apoptosis (Cary et al., 1998). Focal adhesion kinase can stimulate cell migration (Sieg et al., 1999; Ilic et al., 1995) and focal adhesion kinase null embryos exhibit a migration defect (Xu et al., 1996). A dominant-negative focal adhesion kinase leads to cell detachment (Frisch et al., 1996). These results suggest that focal adhesion kinase plays a role in cell adhesion, migration, survival and proliferation. Also, the proliferation of NCI-H1299 cells was stimulated by neurotensin, but inhibited by SR48692, a nonpeptide antagonist. SR48692 inhibited the proliferation of small cell lung cancer, colon and pancreatic cells in vitro and tumors in vivo (Iwase et al., 1996, 1997). Previously, we found that small cell lung cancer cell lines have immunoreactive neurotensin (Moody et al., 1985). Preliminary data (T. Moody, unpublished) indicate that NCI-H1299 cells have immunoreactive neurotensin (0.04 pmol/mg protein). These results suggest that neurotensin may be an autocrine growth factor in NCI-H1299 cells.

In summary, neurotensin binds with high affinity to NCI-H1299 cells, causes tyrosine phosphorylation of focal adhesion kinase and stimulates proliferation. It remains to be determined if neurotensin receptors and focal adhesion kinase are important in lung cancer cellular adhesion and migration.

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