Korean J Physiol Pharmacol
Vol 4: 211-217, June, 2000

Tyrosine Phosphorylation of Paxillin May be Involved in Vascular Smooth Muscle Contraction

Lian-Hua Fang[1], Kyoung-Soo Cho[1], Sang-Jin Lee[2], and Hee-Yul Ahn[1]

Departments of [1]Pharmacology and [2]Physiology, College of Medicine, Chungbuk National University, Chongju 361-763, Korea

Paxillin is a regulatory component of the complex of cytoskeletal proteins that link the actin cytoskeleton to the plasma membrane. However, the role of paxillin during smooth muscle contraction is unclear. We investigated a possible role for the membrane-associated dense plaque protein paxillin in the regulation of contraction in rat aortic vascular smooth muscle. The tyrosine phosphorylation of paxillin, which was increased by norepinephrine, reached a peak level after 1 min stimulation and then decreased with time. However, norepinephrine induced a sustained contraction that reached a steady state 30 min after application. Pretreatment with tyrphostin, an inhibitor of tyrosine kinase, inhibited the tyrosine phosphorylation of paxillin and also the contraction stimulated by norepinephrine. Both inhibitions were concentration-dependent, and the degree of correlation between them was high. These results show that, in rat aortic smooth muscle, tyrosine kinase(s) activated by norepinephrine may phosphorylate the tyrosine residues of paxillin, thereby providing a source of regulation during vascular smooth muscle contraction.

Key Words: Paxillin, Phosphorylation, Tyrosine kinase, Vascular smooth muscle contraction

INTRODUCTION

Phosphorylation of the 20 kDa myosin light chain (MLC) is Ca^{2+} and calmodulin-dependent, and widely believed to be essential for the initiation of smooth muscle contraction (Hartshorne, 1987). Phosphorylation of proteins in tyrosine residues by tyrosine kinase may also contribute to smooth muscle contraction (Di Salvo et al, 1993). The mechanisms that regulate the Ca^{2+} sensitivity of contractile activation in smooth muscle may involve protein tyrosine phosphorylation (Steusloff et al, 1995). The mechanism(s) governing the regulation of smooth muscle contraction by tyrosine kinase is still unclear.

The cytoskeletal proteins vinculin, talin, α-actinin and paxillin, are linker proteins that connect actin filaments of smooth muscle cells to transmembrane integrins, thereby transmitting force across the membrane (Wang et al, 1993; Burridge & Chrzanowska-Wodnicka, 1996). These cytoskeletal proteins have been localized to the membrane associated dense plaque (MADP) sites of normal smooth muscle, as well as to the focal adhesion sites of cultured cells (Burridge et al, 1988; Turner et al, 1990). The assembly of actin stress fibers and the formation of focal adhesion sites has both been correlated with the phosphorylation of MADP proteins (Burn et al, 1988; Turner et al, 1989; Burridge et al, 1992; Romer et al, 1994). Changes in the structure of cytoskeletal proteins may be the basis for the plasticity of the mechanical responses of smooth muscle tissues. Recent studies have shown that serine-threonine phosphorylation of the MADP cytoskeletal protein talin increased in association with force development during isometric contraction of canine tracheal smooth muscle induced by acetylcholine (Pavalko et al, 1995). Like talin, the cytoskeletal protein paxillin binds to vinculin, and may play a regulatory role in linking actin filaments to the plasma membrane (Turner et al, 1990; Burridge et al, 1992; Barry & Critchley, 1994; Hildebrand et al, 1995; Turner et al,

Corresponding to: Hee-Yul Ahn, Department of Pharmacology, College of Medicine, Chungbuk National University, 48 Gaeshin-dong, Chongju 361-763, Korea. (Tel) 82-43-261-2850, (Fax) 82-43-272-1603, (E-mail) hyahn@med.chungbuk.ac.kr

1995). Moreover, tyrosine phosphorylation of paxillin was increased with a time course similar to that of force development during contractile stimulation with acetylcholine, 5-hydroxytryptamine, and KCl in dog tracheal smooth muscle. This suggests that paxillin plays some role in the contractile activation of smooth muscle (Wang et al, 1996). However, the exact relationship between tyrosine phosphorylation of paxillin and the contraction of vascular smooth muscle is not fully understood.

The objective of the present study was to investigate a possible role for the MADP protein paxillin in the regulation of contraction in vascular smooth muscle from rat aorta. The study evaluated the relationship between force generation and paxillin phosphorylation, during muscular contraction and relaxation induced, respectively, by norepinephrine (NE) and tyrphostin, an inhibitor of tyrosine kinase.

METHODS

Tissue preparation and tension measurements

Adult male rats (Sprague-Dawley, 250~350 g) were killed by inhalation of 100% CO_2. Thoracic aortae were carefully isolated and immediately immersed in an ice-cold physiological salt solution (PSS) of the following composition (in mM): NaCl 136.9, KCl 5.4, $NaHCO_3$ 23.8, glucose 5.5, $CaCl_2$ 1.5, $MgCl_2$ 1.0 and ethylenediamine tetra-acetic acid (EDTA) 0.01. Fat and connective tissues were cleaned off the preparation. Endothelium was removed by gently rubbing the intimal space with a cotton swab to avoid the endothelium-dependent relaxation effect of acetylcholine. Helical strips of thoracic aorta (2 mm in width and 10~12 mm in length) were dissected.

Aortic strips were placed in Magnus chambers containing 7 ml of PSS (pH 7.4), aerated continuously with 95% O_2/5% CO_2. A passive tension of 1.0 g was applied initially to each aortic strip, which was then allowed to equilibrate at 37°C for 60 min. Following equilibration, the strips were caused to contract by the addition of KCl-PSS (65.4 mM KCl, made by substituting 60 mM NaCl in normal PSS with equimolar KCl) in order to provide a reference force for standardizing the experimental values. To determine the relaxant effect of the tyrosine kinase inhibitor, tyrphostin was applied 30 min before the administration of NE or after the development of sustained contraction. Changes in muscle tension were recorded isometrically using force-displacement transducers connected to a Gould polygraph (USA).

Cell culture and lysis

Vascular smooth muscle cells (VSMCs) from the thoracic aortae of adult male rats were prepared by digestion using a collagenase-based mixture [0.2% collagenase type I, elastase type II 125 μg/ml, bovine serum albumin 2 mg/ml, soybean trypsin inhibitor type I-S 375 μg/ml, Ham's F12 medium, 15 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)]. The cells were subcultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 units/ml penicillin, 100 μg/ml streptomycin, 8 mM HEPES, and 2 mM L-glutamine. The primary cultured VSMCs were identified by smooth muscle-specific anti-α-actin antibody. Six to nine tissue culture passages of VSMCs were used in these experiments.

VSMCs were subcultured in 100 mm dishes and, at confluence, were maintained in a serum-free medium for 24 h, washed in phosphate-buffered saline (PBS, NaCl 136.9, KCl 2.7, Na_2HPO_4 8.1, KH_2PO_4 1.5; all values in mM, pH 7.4) and then incubated for specifications stated above, with NE at a specified concentration and/or tyrphostin, in a serum-free medium. Stimulation of the VSMCs was terminated by aspiration of the medium and washing with ice-cold PBS containing 1 mM Na_3VO_4. Cells were then lysed, over ice, in a lysis buffer [50 mM tris (hydroxymethyl) aminomethane (Tris) pH 7.4, 1% octylphenoxy polyethoxy ethanol (NP-40), 150 mM NaCl, 0.25% sodium deoxycholate, 1 mM NaF, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM ethylene glycol bis(-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), 10 μg/ml leupeptin, 1 mM Na_3VO_4], scraped, and then sonicated for 20 s at 4°C. The cells were then centrifuged at 15,000 g for 10 min at 4°C. Supernatants were used immediately or stored at −70°C. Protein concentration was determined using a protein assay kit (Bio-Rad).

Immunoprecipitation-immunoblot analysis

Cell extracts were equalized for protein content (200~300 μg) and then precleared with protein G for 1 h with gentle rocking motion at 4°C. After

centrifugation at 15,000 rpm for 10 s, the supernatants were subjected to immunoprecipitation with 4G10 phosphotyrosine antibody for 3 h with gentle rocking at 4°C. Immunocomplexes were recovered by the addition of protein A-agarose, incubated overnight at 4°C and centrifuged at 15,000 rpm for 10 s. The agarose beads were washed with washing solution (20 mM HEPES pH 7.2, 1% NP-40, 50 mM NaF, 10% glycerol, 1 mM PMSF, 1 mM Na_3VO_4, 10 μg/ml leupeptin). The agarose beads were resuspended in a solution of 4× sodium dodecyl sulfate (SDS)-sample buffer and 1× SDS-running buffer (1 : 2 V/V), then heated for 5 min at 95°C. The beads were collected by centrifugation at 15,000 rpm for 10s and the supernatants were then electrophoresed on a 7.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The protein was transferred to nitrocellulose membranes, and subsequent immunoblot analysis was performed with anti-paxillin primary antibody. Enhanced chemiluminescence (ECL) reagents visualized immunoreactive bands. These images were analyzed by densitometry using the Scion image software (NIH).

Reagents

Anti-phosphotyrosine antibody and anti-paxillin (monoclonal IgG1) antibody were obtained from UBI (Lake Placid, NY) and Transduction Laboratories (Lexington, KY), respectively. Immunopure protein G was obtained from Pierce (Rockford, Illinois). Protein A sepharose was obtained from Pharmacia Biotech (Uppsala). Horseradish peroxidase (HRP)-conjugated secondary antibody, rainbow protein molecular weight marker, ECL reagent kit and ECL x-ray film were purchased from Amersham Life Science (Little Chalfont, Buckinghamshire). Pharmacological reagents such as NE, tyrphostin 25, and other chemicals of analytical grade such as glycine, glycerol, Tris base, 2-mercaptoethanol, SDS, acrylamide, bis-acrylamide, ammonium persulfate, N,N,N',N'-tetramethylethylenediamine (TEMED), collagenase, protein standard (BSA) and elastase were obtained from Sigma (St. Louis, MO). DMEM, FBS, penicillin/streptomycin, HEPES, L-glutamine and trypsin-EDTA were purchased from Gibco BRL (Grand Island, NY). Anti-α-actin antibody was obtained from Sigma (St. Louis, MO). Protein assay kit was obtained from Bio-Rad (Richmond, CA).

NE was dissolved in distilled water just before each experiment, and tyrphostin was dissolved in dimethylsulfoxide (DMSO). Stock solutions were diluted so that the final concentration of DMSO in the magnus chambers was $\leq 1\%$.

Statistical analysis

All data are presented as group means standard error of the mean (SEM). Student's *t*-test was used to test for significant differences between means, with $P<0.05$ being considered significant.

RESULTS

After 1 min stimulation with 1 μM NE, the tyrosine

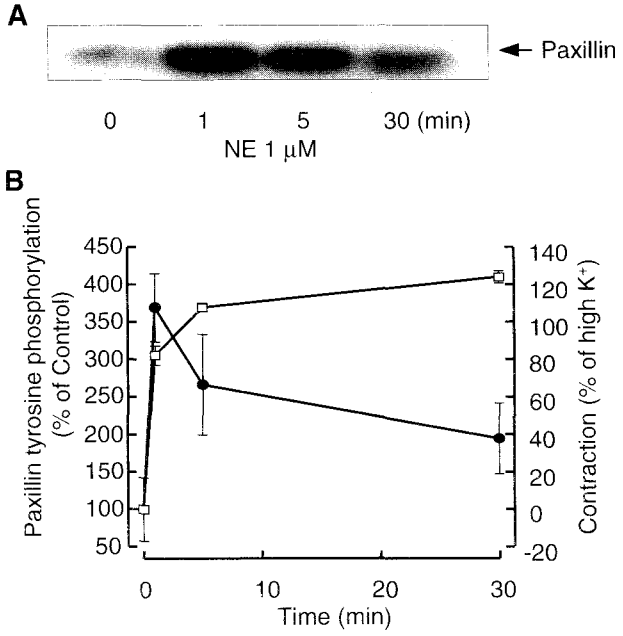

Fig. 1. (A) Immunoblot of tyrosine phosphorylation of paxillin in rat aortic VSMCs stimulated by 1 μM NE. NE increased tyrosine phosphorylation of paxillin maximally at 1 min, followed by a return to control levels over time. This figure is representative of three experiments. (B) Time-course of force development (□) and tyrosine phosphorylation of paxillin (●) of rat thoracic aorta and rat aortic VSMCs in response to 1 μM NE, respectively. Force (right ordinate) is expressed as a % of the contraction in response to KCl-PSS. The absolute magnitude of force in response to KCl-PSS was 0.794± 0.065 g. Tyrosine phosphorylation of paxillin in response to NE was increased transiently with time in contrast with force. Data are expressed as means±SE (n=3~4).

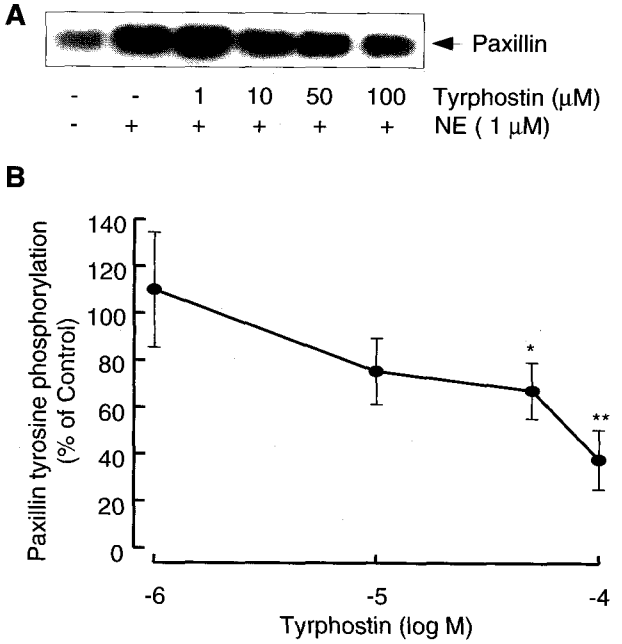

Fig. 2. (A) Immunoblot of tyrosine phosphorylation of paxillin in rat aortic VSMCs stimulated by 1 μM NE. Tyrphostin treatment 30 min prior to application of NE attenuated the tyrosine phosphorylation of paxillin in a concentration-dependent manner. This figure is representative of three experiments. (B) Effect of tyrphostin (1 μM~100 μM) on tyrosine phosphorylation of paxillin in response to 1 μM NE for 1 min in rat aortic VSMCs. Tyrphostin was pretreated 30 min before the application of NE. Tyrphostin reduced, in a concentration-dependent manner, the tyrosine phosphorylation of paxillin. Data are expressed as means±SE (n=3). * and ** denote $P<0.05$ and $P<0.01$ as compared to values obtained during NE stimulation alone, respectively.

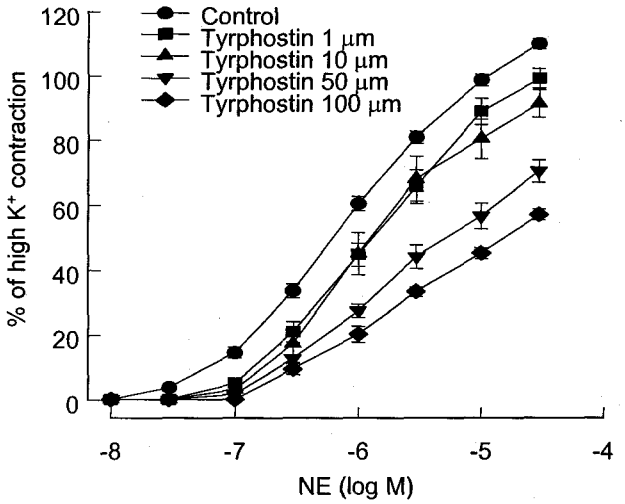

Fig. 3. Concentration-response curves for tyrphostin pretreated to aortic strips in response to NE. Force (left ordinate) is expressed as a % of the contraction in response to KCl-PSS. The absolute magnitude of force in response to KCl-PSS was 0.733±0.053 g. Tyrphostin (1~100 μM) inhibited the contraction in response to NE, concentration-dependently. Data are expressed as means ±SE (n=4).

phosphorylation of paxillin had increased maximally to 3.7±0.5 times (n=3) the level of the control. After 5 min stimulation it was 2.7±0.7 times (n=3) the level of the control. After 30 min stimulation it was 1.9±0.5 times (n=3) the level of the control (Fig. 1A, B). NE stimulation therefore induced a transient increase in the tyrosine phosphorylation of paxillin in VSMCs.

On the other hand, 1 μM NE also induced sustained tension. After 1 min stimulation with 1 μM NE, the contraction was 82.1±5.2% (n=4) of the reference value (based on K^+-induced contraction in rat aorta). After 5 min stimulation with NE, the contraction was 107.6±2.4% (n=4) of the reference value. After 30 min stimulation with NE, the contraction was maximal, at 124.0±3.4% (n=4) of the reference value (Fig. 1B). The maximal increase of tyrosine phosphorylation of paxillin in response to NE preceded the maximal contraction. This indicates that tyrosine phosphorylation of paxillin is involved in the contraction induced by NE in rat aorta. However, although tyrosine phosphorylation of paxillin increased rapidly, followed by a return to the resting levels, the contraction in response to NE was more sustained.

Stimulation with 1 μM NE for 1 min increased the tyrosine phosphorylation of paxillin 2.3±0.1 fold (n=3) over resting values of 1.0±0.1 (n=3). This increase was significant ($P<0.01$). Tyrphostin reduced, in a concentration-dependent manner, the tyrosine phosphorylation of paxillin stimulated by 1 μM NE in VSMCs (Fig. 2A, B). The inhibition of paxillin phosphorylation was 24.7±14.1%, 32.7± 11.9% and 62.0±12.7% in a range from 10 to 100 μM, respectively (n=3). On the other hand, pretreatment with tyrphostin (1 μM~100 μM) for 30 min, inhibited, in a concentration-dependent manner, the smooth muscle contraction in response to 0.01 μM ~30 μM NE (Fig. 3, n=4). We investigated whether the inhibition of tyrphostin on vascular smooth muscle contraction correlated with its effect on the tyrosine phosphorylation of paxillin in response to 1 μM NE. Fig. 4 shows the correlation curve, which

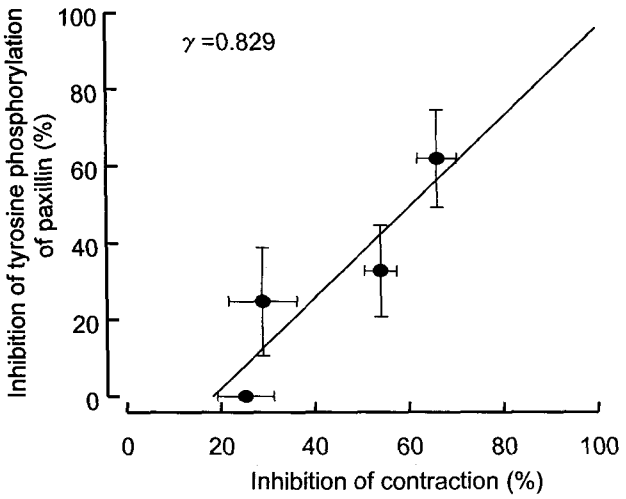

Fig. 4. Correlation, using tyrphostin, between inhibitory effects on contraction and on tyrosine phosphorylation of paxillin in response to 1 μM NE in rat aorta and rat aortic VSMCs. Data are expressed as means±SE (n=3).

corresponds to the relation $y=1.17x-21.65$. The correlation coefficient was 0.829 indicating a positive correlation.

DISCUSSION

It is well known that the phosphorylation of the 20-kDa MLC is not the sole determinant for smooth muscle contraction. In most cases, during stimulation, MLC phosphorylation decreases to suprabasal levels although force is sustained. Thus, force may be maintained by slowly cycling unphosphorylated myosin-actin crossbridges (Dillon et al, 1981). The mechanism responsible for this state of high-force, low phosphorylation, and low energy-consumption (known as the latch state) is not understood. Secondary mechanism(s) for smooth muscle contraction may be involved in the development and/or maintenance of contractile tension. These mechanisms may include: 1) cooperativity in the crossbridge cycle, 2) prolongation of the life-time of the dephosphorylated AM-ADP state by the high affinity of smooth muscle myosin for ADP, 3) an increase in the ratio of crossbridge attachment/detachment constants, and 4) the action of the thin-filament-associated proteins caldesmon and calponin (Somlyo & Somlyo, 1994).

On the other hand, it has been reported that the tyrosine kinase inhibitors, genistein and tyrphostin, inhibited agonist-induced contractions in various tissues of different species, thereby suggesting that tyrosine phosphorylation of some proteins may play a role in agonist-induced contraction (Yang et al, 1992; Di Salvo et al, 1993; Di Salvo et al, 1994). Recently, it was suggested that tyrosine phosphorylation of the MADP protein, paxillin, may play an important role in the regulation of force development in canine tracheal smooth muscle (Pavalko et al, 1995; Wang et al, 1996; Metha et al, 1998).

Our data showed that NE rapidly increased the tyrosine phosphorylation of paxillin in rat aortic vascular smooth muscle (Fig. 1A). Tyrosine kinases are functionally classified into three groups: those associated with cell surface receptors (group 1), the focal adhesion kinases (group 2), and nuclear tyrosine kinases (group 3) (Wang & McMhirter, 1994). NE should activate tyrosine kinase(s) rapidly resulting in a transient tyrosine phosphorylation of paxillin in rat aortic vascular smooth muscle. At present, we do not have data on the specific type of tyrosine kinase that is activated by NE in the rat aorta. However, because tyrosine phosphorylation of paxillin is catalyzed directly by a focal adhesion kinase ($pp125^{FAK}$; see Turner et al, 1993; Bellis et al, 1995), NE may activate $pp125^{FAK}$ by an as yet unknown signal transduction mechanism. Although NE induced a sustained contraction, tyrosine phosphorylation of paxillin occurred transiently in response to NE (Fig. 1B). In rat aortic smooth muscle cells, angiotensin II, endothelin-1, and α-thrombin also caused a rapid tyrosine phosphorylation of paxillin that preceded mitogenesis (Leduc & Meloche, 1995; Turner et al, 1995). However, these results contrast with those obtained from canine tracheal smooth muscle, in which tyrosine phosphorylation of paxillin was increased in association with development of contraction, in response to acetylcholine or 5-hydroxytryptamine (Wang et al, 1996). The difference in time course, between vascular and tracheal smooth muscles, for the tyrosine phosphorylation of paxillin may indicate different roles for paxillin in tracheal and vascular smooth muscles. In rat aorta, the tyrosine phosphorylation of paxillin may be important in the development of the contraction rather than in the maintenance of the contraction. Alternatively, it is possible that the tyrosine phosphorylation of paxillin at 1 minute allows a rearrangement of filament attachments in the submembranous cortex, which allows the maintenance of the force that just devel-

oped. I.e. the tyrosine phosphorylation of paxillin marks the beginning of force maintenance to glue the filaments together in a way that allows force to be maintained even though 20-kDa MLC phosphorylation decreases.

Tyrphostin, an inhibitor of tyrosine kinase, inhibited the tyrosine phosphorylation of paxillin induced by NE in a concentration-dependent manner (Fig. 2A, B). Tyrphostin suppressed the NE-induced contraction in a concentration-dependent manner (Fig. 3). There was a high correlation between the contraction and the tyrosine phosphorylation of paxillin, both in response to NE, under inhibition by tyrphostin (Fig. 4). This indicates that the tyrosine phosphorylation of paxillin is involved in NE-induced contraction in rat aortic smooth muscle.

In conclusion, this study shows that, in rat aorta, the phosphorylation of the cytoskeletal protein paxillin may be associated with the vascular smooth muscle contraction in response to NE. Using tyrphostin, an inhibitor of tyrosine kinase, we showed a high correlation between the inhibition of contraction and the inhibition of the tyrosine phosphorylation of paxillin. This indicates that tyrosine kinase(s) activated by NE may phosphorylate the tyrosine residues of paxillin, thereby providing a source of regulation during vascular smooth muscle contraction.

ACKNOWLEDGMENTS

The authors are extremely grateful to Dr. Kathleen Morgan (Boston Biomedical Research Institute, USA) for her critical reading of this manuscript. This work was supported by grants from the Korea Science and Engineering Foundation (Grant No. of Hee-Yul Ahn: 971-0704-023-2).

REFERENCES

Barry ST, Critchley DR. The RhoA-dependent assembly of focal adhesions in Swiss 3T3 cells is associated with increased tyrosine phosphorylation and the recruitment of both pp125FAK and protein kinase C-delta to focal adhesions. *J Cell Sci* 107: 2033−2045, 1994

Bellis SL, Miller JT, Turner CE. Characterization of tyrosine phosphorylation of paxillin in vitro by focal adhesion kinase. *J Biol Chem* 270: 17437−17441, 1995

Burn P, Kupfer A, Singer SJ. Dynamic membrane-cytoskeletal interactions: specific association of integrin and talin arises in vivo after phorbol ester treatment of peripheral blood lymphocytes. *Proc Natl Acad Sci (USA)* 85: 497−501, 1988

Burridge K, Chrzanowska-Wodnicka M. Focal adhesions, contractility, and signaling. *Annu Rev Cell Dev Biol* 12: 463−518, 1996

Burridge K, Fath K, Kelly T, Nuckolls G, Turner CE. Focal-adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Ann Rev Cell Biol* 4: 487−525, 1988

Burridge K, Turner CE, Romer LH. Tyrosine phosphorylation of paxillin and pp125^{FAK} accompanies cell adhesion to extracellular matrix: a role in cytoskeletal assembly. *J Cell Biol* 119: 893−903, 1992

Dillon PF, Aksoy MO, Driska SP, Murphy RA. Myosin phosphorylation and the cross-bridge cycle in arterial smooth muscle. *Science* 211: 495−497, 1981

Di Salvo J, Pfitzer G, Semenchuk LA. Protein tyrosine phosphorylation, cellular Ca^{2+}, and Ca^{2+} sensitivity for contraction of smooth muscle. *Can J Physiol Pharmacol* 72: 1434−1439, 1994

Di Salvo J, Steusloff A, Semenchuk L, Satoh S, Kolquist K, Pfitzer G. Tyrosine kinase inhibitors suppress agonist-induced contraction in smooth muscle. *Biochem Biophys Res Commun* 190: 968−974, 1993

Hartshorne DJ. In Physiology of the Gastrointestinal Tract. In Johnson LR, editor. *New York: Raven Press.* 423−482, 1987

Hildebrand JD, Schaller MD, Parsons JT. Paxillin, a tyrosine phosphorylated focal adhesion-associated protein binds to the carboxyl terminal domain of focal adhesion kinase. *Mol Biol Cell* 6: 637−647, 1995

Leduc I, Meloche S. Angiotensin II stimulates tyrosine phosphorylation of the focal adhesion-associated protein paxillin in aortic smooth muscle cells. *J Biol Chem* 270: 4401−4404, 1995

Mehta D, Wang Z, Wu M-F, Gunst SJ. Relationship between paxillin and myosin phosphorylation during muscarinic stimulation of smooth muscle. *Am J Physiol* 274: C741−C747, 1998

Pavalko FM, Adam LP, Wu MF, Walker TL, Gunst SJ. Phosphorylation of dense-plaque proteins talin and paxillin during tracheal smooth muscle contraction. *Am J Physiol* 268: C563−C571, 1995

Romer LH, McLean N, Turner CE, Burridge K. Tyrosine kinase activity, cytoskeletal organization, and motility in human vascular endothelial cells. *Mol Biol Cell* 5: 349−361, 1994

Somlyo AP, Somlyo AV. Signal transduction and regulation in smooth muscle. *Nature* 372: 231−236, 1994

Steusloff A, Paul E, Semenchuk LA, Di Salvo J, Pfitzer G. Modulation of Ca^{2+} sensitivity in smooth muscle

by genistein and protein tyrosine phosphorylation. *Arch Biochem Biophys* 320: 236−242, 1995

Turner CE, Glenney JR jr, Burridge K. Paxillin: a new vinculin-binding protein present in focal adhesions. *J Cell Biol* 111: 1059−1068, 1990

Turner CE, Pavalko FM, Burridge K. The role of phosphorylation and limited proteolytic cleavage of talin and vinculin in the disruption of focal adhesion integrity. *J Biol Chem* 264: 11938−11944, 1989

Turner CE, Schaller MD, Parsons JT. Tyrosine phosphorylation of the focal adhesion kinase pp125^{FAK} during development: relation to paxillin. *J Cell Sci* 105: 637−645, 1993

Turner CE, Pietras KM, Taylor DS, Molloy CJ. Angiotensin II stimulation of rapid paxillin tyrosine phosphorylation correlates with the formation of focal adhesions in rat aortic smooth muscle cells. *J Cell Sci* 108: 333−342, 1995

Wang N, Butler JP, Ingber DE. Mechanotransduction across the cell surface and through the cytoskeleton. *Science* 260: 1124−1127, 1993

Wang Z, Pavalko FM, Gunst SJ. Tyrosine phosphorylation of the dense plaque protein paxillin is regulated during smooth muscle contraction. *Am J Physiol* 271: C1594−C1602, 1996

Wang JYJ, McMhirter JR. Tyrosine-kinase-dependent signaling pathways. *Trends Cardiovasc Med* 4: 264−270, 1994

Yang SG, Saifeddine M, Hollenberg MD. Tyrosine kinase inhibitors and the contractile action of epidermal growth factorurogastrone and other agonists in gastric smooth muscle. *Can J Physiol Pharmacol* 70: 85−93, 1992