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Role of Non-receptor Protein Tyrosine Kinases During Phospholipase C- γ 1 Related Uterine Contractions in the Rat

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Abstract

Activated phospholipase C γ 1 (PLC- γ 1), produced in response to tyrosine phosphorylation, appears to play an important role during uterine contractions. These studies sought to determine which non-receptor protein tyrosine kinases (PTKs) are involved in the tyrosine phosphorylation and activation of PLC- γ 1 in uterine tissue from the rat. *In vitro* uterine contraction studies were performed utilizing isoform specific PTK inhibitors. Western blots were performed utilizing antibodies to phosphotyrosine-PLC- γ 1, total PLC- γ 1, c-Src kinase and Lck kinase. Spontaneous, stretch-stimulated, and bpV(phen) (a tyrosine phosphatase inhibitor) enhanced uterine contractions were significantly suppressed in response to Damnacanthal (a Lck kinase inhibitor) and PP1 (a c-Src kinase inhibitor); whereas, several other PTK isoform inhibitors had no significant effect. Damnacanthal and PP1 also significantly suppressed bpV(phen)-enhanced tyrosine phosphorylation of PLC- γ 1 compared to other PTK isoform inhibitors. Western blots confirmed expression of the Lck and c-Src kinases in uterine tissue. In conclusion, the Lck and c-Src kinases appear to play an important role in regulating tyrosine phosphorylation of PLC- γ 1 and contractile activity in the rat uterus.

Keywords

Lck Kinase; c-Src Kinases; Phospholipase C- γ 1; Phasic Myometrial Contractions; Uterine Stretch

Introduction

Activation of phospholipase C- γ (PLC γ) results in inositol trisphosphate (IP₃) generation, stimulation of the phosphatidylinositol (PI) signaling pathway, and mobilization of intracellular calcium in various cell types including in uterine myocytes¹. Two isoforms of PLC γ have been previously reported: the PLC γ 1 isoform is expressed in a wide range of cell types and animal tissues; whereas, the PLC γ 2 isoform has been identified mainly in white blood cells and lymphoid tissues^{2,3}. Western blot, reverse transcriptase polymerase chain reaction (RT-PCR), and immunohistochemical studies previously reported by our laboratory have confirmed the expression of both of these PLC γ isoforms in pregnant and non-pregnant rat myometrial tissue^{4,5}. These previous studies using rat uterine tissue were consistent with those reported by Phaneuf et al.⁶ who utilized Western blots to demonstrate the expression of PLC γ 1 and PLC γ 2 in human myometrial cells.

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PLC γ activation occurs by phosphorylation of tyrosine #783 in response to various membrane receptor tyrosine kinases and non-receptor protein tyrosine kinases (PTKs) ^{2, 3}. Members of the Src family of non-receptor protein tyrosine kinases have been reported to produce tyrosine phosphorylation of PLC γ 1 in various smooth muscle types, including in myometrium. Schmitz et al. ⁷ have reported that angiotensin II stimulates tyrosine phosphorylation of PLC γ through the activation of c-Src in vascular smooth muscle cells. Boulven et al. ⁸ demonstrated the ability of c-Src to generate phosphotyrosine-PLC γ 1 in rat myometrial cells; an effect that was prevented by pretreatment of the tissue with the tyrosine kinase inhibitors genistein and PP1 (4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine). In a previous report, we utilized bpV(phen) (potassium bisperoxo (1,10 phenanthroline) oxovanadate) to demonstrate the role of PLC γ 1 and its tyrosine phosphorylation during phasic contractions of rat uterine tissue ¹.

To date, at least 9 members of the Src family of non-receptor PTKs have been demonstrated in vertebrate cells. These Src family kinase isoforms include c-Src (the original member) along with the Blk, Fgr, Fyn, Hck, Lck, Lyn, Yes and Yrk isoforms; all have a common molecular structure, conserved Src-homology 2 (SH2) and Src-homology 3 (SH3) peptide domains, and similar molecular weights in the 52–62 kD range ^{9, 10}. The Src kinases are activated through dephosphorylation of a tyrosine residue at their carboxy-terminal ends and protein-protein interactions (at their SH2 and SH3 domains), resulting in exposure of the catalytic domain. Several non-receptor PTKs, including c-Src, Lck, Fyn, Lyn, Hck and Syk (a non-Src family kinase), have been previously reported to produce tyrosine phosphorylation of PLC γ in various cell types ^{11–13}. The goal of the present study was to determine if any of these PTKs play a role during tyrosine phosphorylation of PLC γ 1 and the generation of spontaneous and bpV(phen)-enhanced phasic contractions of the rat uterus. In addition, we sought to determine if these PTK signaling events also contribute to the mechanisms underlying the stretch-stimulated phasic uterine contractions.

Materials & Methods

Uterine and other tissues were obtained for these studies from nonpregnant and timed-pregnant Sprague-Dawley rats using a protocol approved by the Animal Care and Utilization Committee at the University of Vermont College of Medicine. For the in vitro isometric contraction studies, uterine tissue was obtained from proestrus/estrus rats. These studies were performed using longitudinal segments of uterine tissue (6–8 mm relaxed length) in 3 mL muscle baths containing Earle's balanced salt solution (EBSS) at 37° C as previously reported by our laboratory ¹. Some contraction studies were performed using 20 μ M potassium bisperoxo (1,10 phenanthroline) oxovanadate (bpV(phen)) (Calbiochem, San Diego, CA); a previously reported inhibitor of protein tyrosine phosphatases ¹. Other contraction studies were performed with and without the addition of previously reported PTK inhibitors. PP1 (4-amino-5-(4-methylphenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine; Biomol International, L.P. Plymouth Meeting, PA) or PP2 (4-amino-5-(4-chlorophenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine; Calbiochem, San Diego, CA) (60 μ M) were used to selectively inhibit c-Src kinase activity ^{8, 14, 15}; Damnacanthal (Calbiochem, San Diego, CA) (60 μ M) was used to inhibit Lck kinase activity ¹⁶; and Piceatannol (Calbiochem, San Diego, CA) (60 μ M) to inhibit Syk kinase activity ¹⁷. Studies were also performed using SU6656 (Calbiochem, San Diego, CA) (100 μ M), an inhibitor of the Fyn, Yes and Lyn kinase isoforms, and which also weakly inhibits c-Src kinase ^{15, 18}. Control studies were performed using comparable volumes of vehicle alone. The PTK inhibitor concentrations were based on the in vitro concentration-response effects of these inhibitors on contractile activity of uterine strips; the selected inhibitor concentrations were at or above the concentrations used in the previously referenced studies in an effort to optimize potential inhibitory effects.

Additional studies were performed to assess the effects of Damnacanthal, PP1, Piceatannol and PAO (phenylarsine oxide, a PLC γ inhibitor¹) on uterine stretch. For these studies, the tissue was preincubated with these reagents for 20 minutes followed by rapid stretch to 4 grams of tension; the resulting contractile activity was then compared to that observed for uterine strips pretreated with vehicle (dimethylsulfoxide (DMSO)) alone. As previously reported from our laboratory¹, the analog contraction data were computer digitized, analyzed to determine the total contractile area in selected 5-minute intervals, normalized for tissue cross-sectional area, and reported as the percent of spontaneous contractile activity (or for the stretch studies: as contractile activity in grams tension generated per milligram tissue per minute).

For the Western blots, uteri and other rat tissues were homogenized in a protease/phosphatase inhibitor solution (containing 50 mM Tris, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 5 μ g/mL aprotinin, 3 μ g/mL leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate). Homogenate solutions were centrifuged at 800 X g to remove the cellular debris; and the protein concentrations of the supernatant solutions (crude tissue homogenates) were determined by BCA protein assay. Subsequently, 50 μ g protein aliquots were resolved using 10% SDS-PAGE gels and the Bio-Rad Mini-PROTEAN 3 Electrophoresis system (Bio-Rad Laboratories, Hercules, CA). The resolved proteins were then electrophoretically transferred to nitrocellulose membranes. The nitrocellulose membranes were blocked with 5% powdered milk in Tween-20/Tris buffered saline (TTBS), then incubated overnight in the primary antibody solution containing anti-phosphotyrosine-PLC γ 1 polyclonal antibodies (Cell Signaling Technology, Danvers, MA), or with anti-PLC γ 1 monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for total PLC γ 1 protein. Additional Western blots were performed using anti-Src (Calbiochem, San Diego, CA) and anti-Lck (Santa Cruz Biotechnology; Santa Cruz, CA) monoclonal antibodies. Visualization of the chemiluminescent protein bands was performed by exposing Hyperfilm (Amersham, Piscataway, NJ) to the nitrocellulose membranes. The relative density of the bands was then determined using the Kodak 1-D Digital Science Electrophoresis Documentation and Analysis System (Eastman Kodak Co, Rochester, NY).

Statistical analyses for these studies were performed using the t-test, Mann-Whitney Rank Sum test, or the Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks, followed by multiple comparisons tests (including the Student-Newman-Keuls or Dunn test) where appropriate. Statistical significance was considered attained when $p < 0.05$.

Results

In vitro contraction studies

Spontaneous uterine contractions were significantly enhanced in response to bpV(phen), a protein tyrosine phosphatase inhibitor, as observed in the vehicle (DMSO) control strips in Figure 1. Damnacanthal (an inhibitor of the Lck kinase isoform) significantly suppressed spontaneous and bpV(phen)-enhanced uterine contractile activity ($p < 0.01$, $N = 7-8$) (Figure 1A and 2). PP1 (an inhibitor of c-Src kinase) also significantly suppressed spontaneous and bpV(phen)-enhanced contractile activity ($p < 0.05$, $N = 8-9$) (Figure 1B and 2). In contrast, Piceatannol (a Syk kinase inhibitor) had no effect on spontaneous or bpV(phen)-enhanced contractile activity (Figure 2). Similarly, SU6656 (an inhibitor of the Fyn, Yes and Lyn kinase isoforms, and a weak inhibitor for c-Src) failed to suppress spontaneous and bpV(phen)-enhanced contractions (Figure 2). These data (as observed in Table I) support a potential role for the Lck and c-Src kinases expressed in the uterus during spontaneous and bpV(phen)-enhanced phasic uterine contractions. As observed in Figure 3 (top panel), acute stretch of uterine strips results in the rapid generation of robust phasic contractions; this

response was significantly suppressed in those strips pretreated with the Lck inhibitor Damnacanthal ($p < 0.05$, $N = 12$) and the c-Src inhibitor PP1 ($p < 0.05$, $N = 5$) (Figure 3B). In contrast, pretreatment Piceatannol failed to suppress stretch stimulated contractile activity, thereby suggesting that Syk kinase does not play an important role during stretch mediated contractions. SU6656 also failed to suppress the stretch-stimulated contractile activity. Stretch studies performed using PAO (a PLC- γ inhibitor that has been previously reported to inhibit bpV(phen)-enhanced myometrial contractions¹) also significantly suppressed stretch-stimulated contractile activity as shown in Figure 3B ($p < 0.01$, $N = 6$). In contrast, pretreatment with bpV(phen) significantly enhanced stretch-stimulated contractions ($p < 0.01$, $N = 8$) (Figure 3B); a result that is consistent with its ability to maintain PLC- γ phosphorylation leading to enhanced spontaneous contractions.

Western blots for phosphotyrosine PLC- γ 1

Western blots were performed using proteins isolated from uterine tissue pretreated for 20 minutes with PTK inhibitors, followed by treatment with 10 μ M bpV(phen) for 10 minutes. These studies demonstrated that PP1 or Damnacanthal significantly suppressed bpV(phen)-enhanced tyrosine phosphorylation (Figure 4). Interestingly, SU6656 also produced a modest, but significant reduction of bpV(phen)-enhanced tyrosine phosphorylation of PLC- γ 1. In contrast, PP2 (another c-Src kinase inhibitor) failed to suppress phosphotyrosine-PLC- γ 1 levels in uterine tissue during this series of experiments (Figure 4). Studies performed using Piceatannol similarly failed to demonstrate significant suppression of bpV(phen)-enhanced tyrosine phosphorylation of PLC- γ 1 in uterine tissue (Figure 4).

Western blots for c-Src and Lck Expression

Additional Western blots were performed to qualitatively compare the expression of c-Src and Lck kinases in the uterus to multiple other rat tissues. As observed in Figure 5, these studies confirmed robust expression of c-Src kinase in uterine tissue at levels comparable to several other rat tissues including lung, spleen, heart, kidney, small intestine and skeletal muscle. These Western blots also demonstrated a similar pattern of expression of the Lck kinase in uterine tissue compared to the other rat tissues.

Comments

Confirming the important role for two members of the Src-family of non-receptor PTKs, these studies have demonstrated a significant decrease in spontaneous and bpV(phen)-enhanced contractions of rat uterine strips when treated with isoform specific inhibitors for the Lck and c-Src kinases. In contrast, inhibitors for other members of the Src-family and for Syk kinase did not significantly affect this contractile activity. Experiments performed to evaluate the effects of these PTK inhibitors on stretch-stimulated uterine contractions also demonstrated a significant decrease in contractile activity in response to the Lck and c-Src kinase inhibitors. The semi-quantitative tyrosine phosphorylation studies demonstrating a significant decrease in PLC γ 1-phosphotyrosine levels provide support for the proposed mechanistic relationship between these PTKs and uterine contractions (see Figure 6). Although only descriptive, the Western blots demonstrating the robust expression of c-Src and Lck kinases in uterine tissue suggest a potential role for these two non-receptor PTKs during uterine function.

The Src kinases are a family of non-receptor protein tyrosine kinases consisting of at least 9 members. The members of this family are 52–62 kD proteins comprised of six structural domains including the Src homology domains; i.e. the SH2 domain and the SH3 domain^{9, 10}. Src-family tyrosine kinases are found associated with cell membranes, appear to produce their downstream effects through tyrosine phosphorylation, and have been

implicated in the regulation of cell proliferation, differentiation, motility and adhesion. Src-family kinases function in pathways leading to PLC activation via direct phosphorylation of PLC γ itself and/or of linker proteins associated with PLC γ ¹⁹. Tyrosine kinase activity of Src proteins is regulated by their phosphorylation status: phosphorylation of Tyrosine residues #527 or #530 results in an interaction between the carboxy terminus and the SH2 domain of Src protein, thereby suppressing its tyrosine kinase activity. Conversely, dephosphorylation of this site relieves this inhibition and Src kinase becomes active. In addition, phosphorylation of Tyrosine residues #416 or #419 of c-Src results in an increase in its tyrosine kinase activity¹⁰.

Lymphocyte specific kinase (Lck) is a Src-family tyrosine kinase isoform first demonstrated to be expressed in T-lymphocytes and implicated in the regulation of T cell receptor expression and in T cell selection²⁰. Like other Src-family kinases, the Lck isoform is regulated by tyrosine phosphorylation; the specific regulatory site is Tyrosine residue #505²⁰. Ramos-Morales, et al.²¹ have demonstrated that the SH2 domain of Lck associates with other tyrosine and serine/threonine kinases; these phenomena suggest that tyrosine-phosphorylated Lck may act as a docking point for many other proteins that contain this SH2 domain resulting in complexes important in signal transduction. Lck has been implicated in PLC γ activation. Ozdener et al.²² have suggested that activation of PLC γ -2 by Lck is mediated by phosphorylation of tyrosines located in the SH2–SH3 linker region. Veri et al.²³ have reported that Lck is necessary for phosphorylation of PLC γ -1 in lipid rafts. The results in our report suggest that the Lck kinases are involved in the phosphorylation of PLC γ -1 in the rat uterine myocytes.

Src kinases are widely expressed in many cell types, including smooth muscle cells. Not surprisingly, expression of Src kinase isoforms varies within the family; some kinases (Src, Fyn, Yes and Yrk) are expressed ubiquitously while the expression of other kinases (Lyn, Hck, Fgr, Blk and Lck) has been reported to be more limited²⁴. Our Western blot studies have demonstrated the robust expression of the c-Src and Lck kinase isoforms in several rat tissues including the uterus. Hollenberg has suggested that tyrosine kinase pathways may play a role in the regulation of smooth muscle contraction²⁵. The c-Src kinase isoform is highly expressed in vascular smooth muscle cells²⁶, is present in unstimulated smooth muscle, and c-Src constitutively modulates Ca²⁺ channels in smooth muscle²⁷. Recently, Che et al.²⁸ have demonstrated that Src family kinases are involved in angiotensin II-stimulated arterial contractions in rat. In 1996, Palmier et al.²⁹ showed that pervanadate (a tyrosine phosphatase inhibitor) enhances myometrial contractions through PLC γ -1 activation; these investigators also showed that inhibition of Src kinase activity suppressed this effect, thereby providing support for the hypothesis that Src activation is responsible for PLC γ -1 related myometrial contractions. In 2002, Boulven et al.⁸ demonstrated that Src kinase activity was enhanced by treatment with pervanadate. These investigators also demonstrated that tyrosine phosphorylation of PLC- γ 1 along with IP₃ production were suppressed in response to treatment of rat myometrium with PP1; these observations led these investigators to conclude that c-Src kinase is responsible for the PLC- γ 1 signaling events in rat myometrium. In our current study, suppression of contractile activity in response to inhibition of both the c-Src and Lck kinases suggests that both of these PTKs are involved in the signaling pathway contributing to uterine contractions. In contrast, other members of the Src-family and the Syk kinase (a non-Src family PTK) do not appear to play a role in spontaneous, stretch, or bpV(phen)-enhanced myometrial contractions despite their potential ability to tyrosine phosphorylate PLC- γ 1.

Previous studies have suggested that activated ion channels are directly involved in stretch-induced contractions in smooth muscle³⁰, and that stretch-induced contractions in uterine tissue are dependent on an influx of Ca²⁺ from the extracellular space³¹. Modulation of

voltage dependent channels leads to the influx of Ca^{2+} contributing to stretch induced contraction; these phenomena also appear to involve IP_3 , diacylglycerol and/or protein kinase C activation³². In 1994, Tanaka et al.³³ demonstrated that the IP_3 concentration in coronary arteries was three fold higher than basal after undergoing stretch; that a PLC inhibitor abolished stretch-induced IP_3 production; and that this PLC inhibitor partially suppressed stretch induced contraction. These investigators suggested that stretching may increase IP_3 formation through a mechanism involving PLC activation³³. A report by Matsumoto et al.³⁴ has demonstrated that stretch induced contractions are related to Ca^{2+} entry through cation channels, that PLC appears to be activated by stretch, that PLC is involved in the cellular response to stretch, and that stretch induced PLC activation is linked to Ca^{2+} influx through cation channels. Oeckler et al.³⁵ have reported the involvement of Src family kinases during stretch induced contraction of the bovine coronary artery. In 2002, Oldenhof et al.³⁶ showed that stretching rat myometrial cells induced rapid and transient activation of mitogen-activated protein kinase (MAPK), extracellular-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK); that upstream tyrosine kinase activity is necessary in the pathway leading to stretch induced MAPK activation; and that Src kinase activity may be involved in MAPK activation. In a recently published report, Li et al.³⁷ have described stretch-induced Src kinase activity, increased focal adhesion signaling including ERK phosphorylation, and enhanced in vitro contractile activity of myometrial smooth muscle from near term pregnant rats. All of these effects of myometrial stretch were markedly suppressed by inhibition of Src kinase activity. Consistent with this report by Li et al.³⁷, our current report has demonstrated that two Src-family PTKs (i.e. c-Src and Lck kinase) appear to be necessary and important mediators in the signaling pathway leading to stretch-induced myometrial contractions. These observations have provided support for the hypothesis that stretch-induced myometrial contractions are mediated, in part, through activation of $\text{PLC}\gamma$ signaling pathway as demonstrated in Figure 6.

In summary, these studies have provided insight into the molecular mechanisms occurring during myometrial contractions of the rat uterus, and more specifically they have helped to elucidate the signaling pathway related to phosphotyrosine- $\text{PLC}\gamma$ -1 mediated contractions of the uterus. In this report, we have demonstrated that the Src family kinases c-Src and Lck are expressed in rat myometrium, and appear to play a role during spontaneous, bpV(phen) (a tyrosine phosphatase inhibitor) enhanced, and stretch-induced myometrial contractions. Understanding of these intracellular signaling pathways will ultimately allow the development of more effective pharmaceutical modalities to treat abnormalities of human parturition leading to ineffective contractions (that result in the increasing need for Cesarean section deliveries) and/or inappropriate contractions (that result in preterm labor and delivery).

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Fig 1A

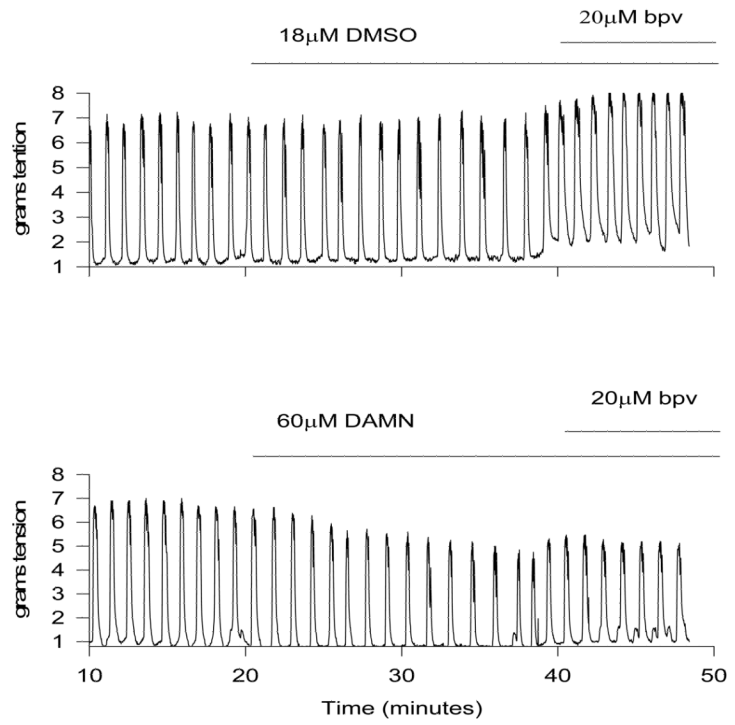


Fig 1B

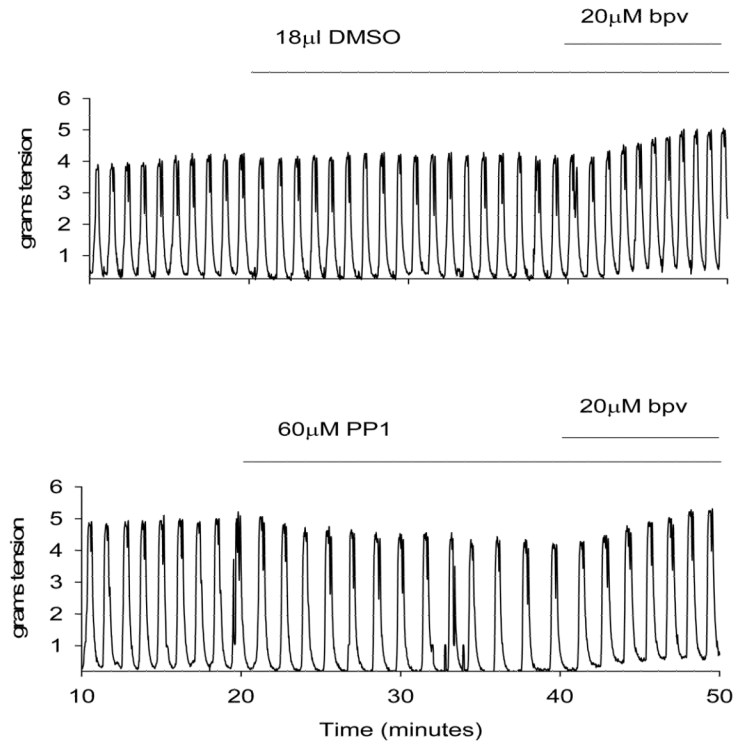


Figure 1.

A. In vitro contraction study demonstrating the effect of vehicle (DMSO), Damnacanthal (DAMN, a Lck kinase inhibitor), and bpV(phen) (a protein phosphatase inhibitor) on spontaneous contractions of the proestrus/estrus rat uterine strips. The uterine strips were incubated with these agents for the time periods indicated by the horizontal bars. Contractile activity reported in grams of tension generated. **B.** In vitro contraction study demonstrating the effect of vehicle (DMSO), PP1 (a c-Src kinase inhibitor) and bpV(phen) on contractile activity. The uterine strips were incubated with these agents for the time periods indicated by the horizontal bars. Contractile activity reported in grams of tension generated.

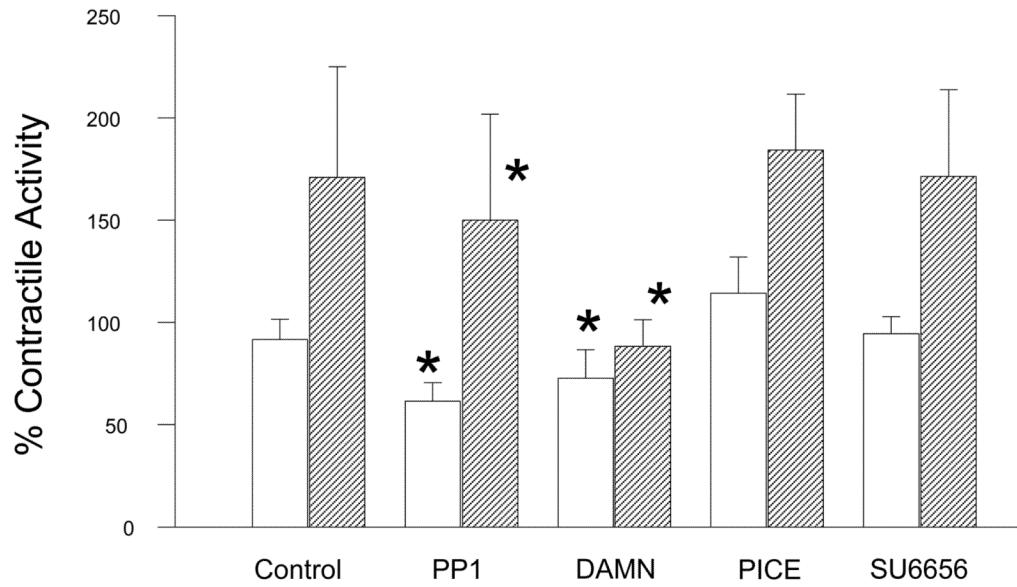


Figure 2.

Bar graph demonstrating the effects of PP1 (a c-Src kinase inhibitor), Damnacanthal (DAMN, a Lck kinase inhibitor), Piceatannol (PICE, a Syk kinase inhibitor) and SU6656 (an inhibitor of the Fyn, Yes, and Lyn kinases, and a weak c-Src inhibitor) on spontaneous (open bars) and bpV(phen)-enhanced (hatched bars) contractile activity. Contractile activity reported as the percent spontaneous (pretreatment) contractile activity. Each bar = mean \pm S.D. N = 4–9 experiments. (*) $p < 0.05$ for comparison to vehicle treated strips using the t-test or Mann-Whitney Rank Sum test.

Fig. 3A

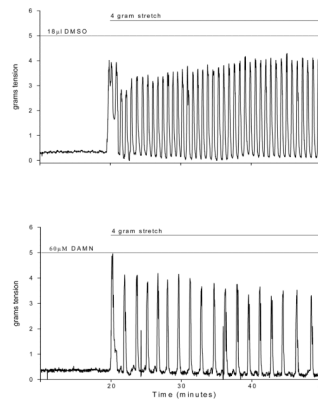
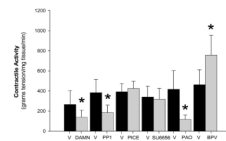


Fig. 3B

**Figure 3.**

A. In vitro contraction study demonstrating the effect of vehicle (DMSO) and Damnacanthal (DAMN, a Lck kinase inhibitor) on stretch-stimulated contractile activity. The uterine strips were incubated with these agents for the time periods indicated by the horizontal bars and then stretched to 4 grams of tension, as noted. Contractile activity recorded and reported in grams of tension generated. **B.** Bar graph demonstrating the effects of Damnacanthal (DAMN, a Lck inhibitor), PP1 (a c-Src inhibitor), Piceatannol (PICE, a Syk inhibitor), SU6656 (an inhibitor of the Fyn, Yes, and Lyn kinases, and a weak c-Src inhibitor), PAO (a PLC γ inhibitor) and bpV(phen) (a protein phosphatase inhibitor) on stretch stimulated contractions (gray bars) compared to vehicle treated strips (black bars). Contractile activity in grams tension/mg tissue/minute. Each bar = mean \pm S.D. N = 5–12 experiments. (*) p < 0.05 for comparison to vehicle treated strips using the t-test or Mann-Whitney Rank Sum test.

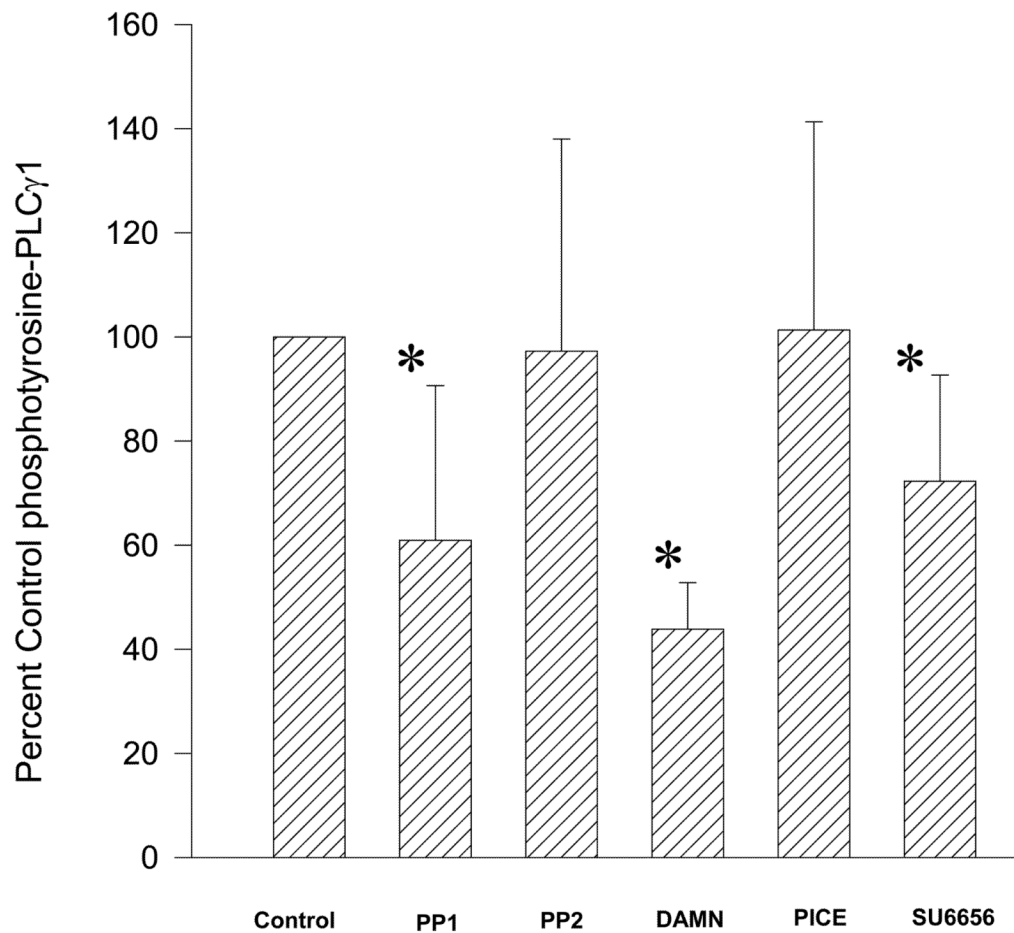


Figure 4.

Bar graph demonstrating the effects of PP1 and PP2 (both are c-Src kinase inhibitors), Damnacanthal (DAMN, a Lck kinase inhibitor), Piceatannol (PICE, a Syk kinase inhibitor) and SU6656 (an inhibitor of the Fyn, Yes, and Lyn kinases, and a weak c-Src inhibitor) on phosphotyrosine PLC γ 1 levels. The band densities were normalized to total PLC γ 1 and reported as the percent of the control (vehicle) band densities. Each bar = mean \pm S.D. N = 3–13 experiments. (*) $p < 0.05$ for comparison to vehicle treated strips using ANOVA on Ranks.

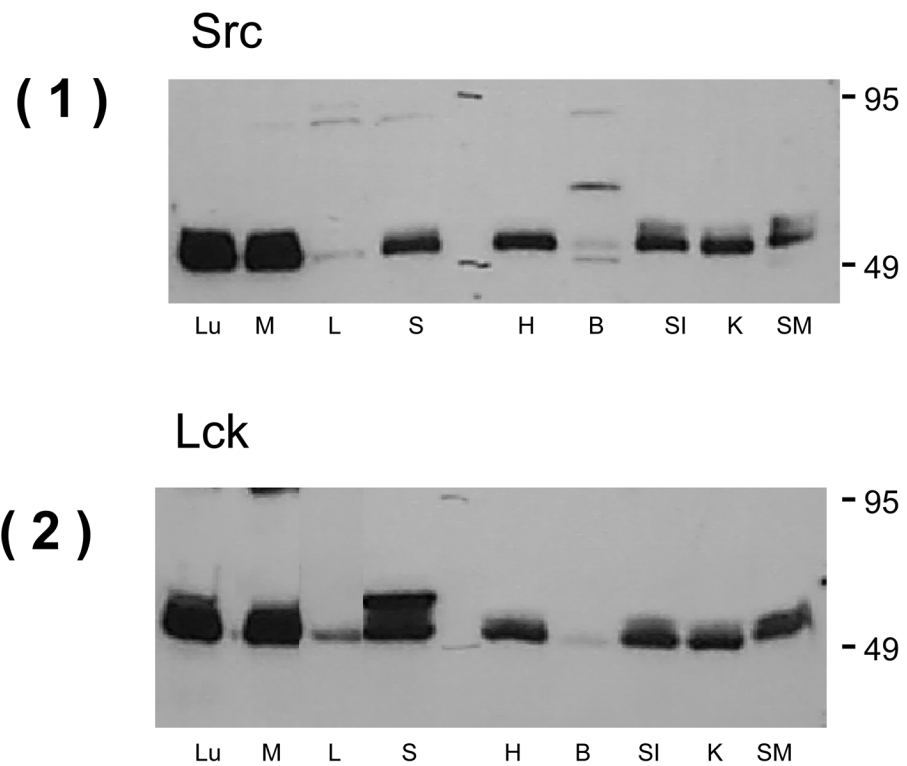


Figure 5.

(1) Western blots demonstrating c-Src kinase expression in several rat tissue (Lu = lung, M = myometrium (uterus), L = liver, S = spleen, H = heart, B = brain, SI = small intestine, K = kidney, and SM = skeletal muscle). **(2)** Another Western blot demonstrating Lck kinase expression in the spectrum of rat tissues noted above.

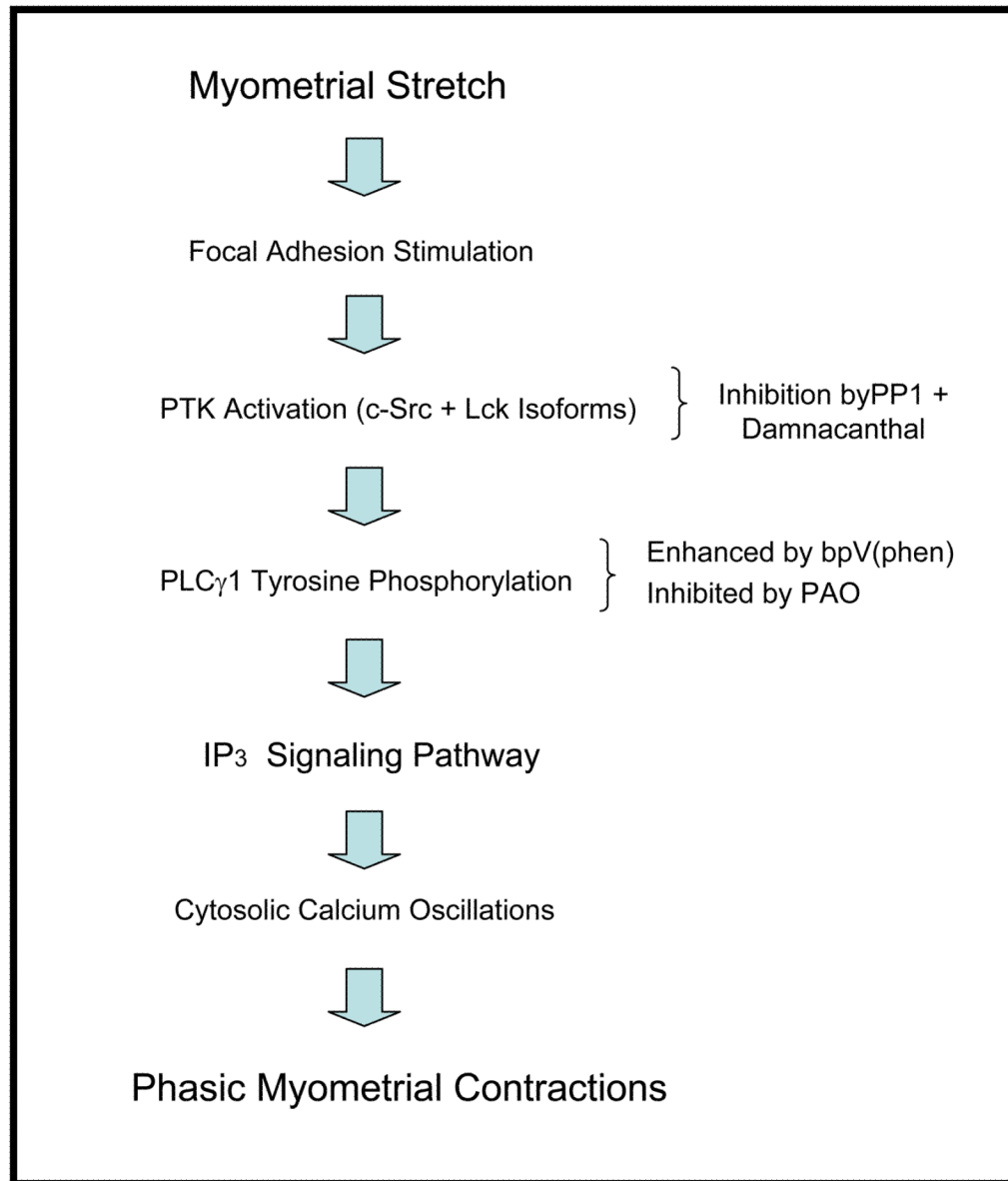


Figure 6. Model demonstrating the hypothesis that stretch-stimulated phasic contractions are mediated through activation of protein tyrosine kinase (PTK) activity produced by c-Src and Lck kinases (or inhibition in response to PP1 and Damnacanthal). This results in tyrosine phosphorylation of phospholipase C γ 1 (PLC γ 1) (which is enhanced by bpV(phen) or inhibited by PAO) leading to activation of the inositol trisphosphate (IP $_3$) signaling pathway and ultimately resulting in cytosolic calcium oscillations that drive phasic myometrial contractions.

Table 1

	Spont. CTX + Vehicle	Spont CTX + Agent	bpV(phen) + Vehicle	bpV(phen) + Agent	(N)
Damnacanthal	97.9 ± 10.8	72.7 ± 14.0*	157.5 ± 27.0	88.3 ± 13.0*	(7-8)
PP1	85.8 ± 9.0	61.5 ± 9.1*	214.5 ± 69.2	150.0 ± 51.9*	(8-9)
Piceatannol	93.8 ± 11.7	114.2 ± 17.9	165.1 ± 35.2	184.4 ± 27.2	(4)
SU6656	90.8 ± 4.4	94.5 ± 8.4	132.4 ± 24.6	171.3 ± 42.4	(6)

Notes:

Spont. CTX = spontaneous contractions

Mean ± standard deviation

(*) = p<0.05 compared to vehicle treated strips