BASIC SCIENCE: OBSTETRICS

The role of phospholipase Cγ1 tyrosine phosphorylation during phasic myometrial contractions

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OBJECTIVE: Phospholipase Cγ1 (PLCγ1) is expressed in myometrium and is activated by tyrosine phosphorylation. These studies sought to determine the association between PLCγ1 tyrosine phosphorylation and spontaneous uterine contractions.

STUDY DESIGN: In vitro contraction studies were performed with spontaneously contracting rat uterine strips along with strips that were treated with potassium bisperoxo(1,10 phenanthroline)oxovanadate (bpV(phen), a protein tyrosine phosphatase inhibitor. Additional studies were performed with phenylarsine oxide (a PLCγ inhibitor) and other inhibitors. Western blots were performed to determine the phosphotyrosine PLCγ1 levels.

RESULTS: Spontaneous contractile activity and tyrosine phosphorylation of PLCγ1 (but not PLCγ2) were increased significantly in response to bpV(phen); in contrast, oxytocin and thrombin produced comparable contractile activity but did not alter phosphotyrosine-PLCγ1. Phenylarsine oxide and neomycin significantly decrease bpV-(phen)-stimulated contractions and PLCγ1 tyrosine phosphorylation; other inhibitors only suppressed contractions.

CONCLUSION: These studies support the hypothesis that spontaneous myometrial contractions are associated with tyrosine phosphorylation of PLCγ1; both of which are further enhanced by the inhibition of protein tyrosine phosphatase activity.

Key words: Myometrial contraction, phospholipase Cγ1 (PLCγ1), rat uterus, tyrosine phosphorylation

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Myometrial contractile activity is mediated in part by the stimulation of the phosphatidylinositol (PI) signaling pathway through activation of phospholipase C (PLC) enzyme activity.[1] Several PLC isoforms have been demonstrated in myometrial tissue. The PLCβ isoforms are activated through G-protein–coupled receptor stimulation by uterotonic agonist, such as oxytocin and prostaglandin F2α; in contrast, the activation mechanisms for the PLCδ isoforms remain poorly defined.[1,2] The PLCγ isoforms are the only isoforms that contain Src-homology 2 domains, thereby allowing activation by receptor and nonreceptor protein tyrosine kinases.[1,2] Western blot studies by our laboratory in 1992 were the first to demonstrate the expression of the PLCγ isoform in pregnant rat myometrial tissue.[3] A subsequent study that used reverse transcriptase–polymerase chain reaction has allowed us to confirm PLCγ1 and PLCγ2 messenger RNA expression in pregnant rat myometrium.[4] More recently, we have used immunohistochemical techniques to confirm the expression of these 2 PLC isoforms in smooth muscle cells of the pregnant rat uterus.[5] Our studies with rat uterine tissue are consistent with those reported by Phaneuf et al,[6] who used Western blots to confirm the expression of PLCγ1 and PLCγ2 protein in human myometrial cells.

The goal of the present study was to test the hypothesis that constitutive and enhanced tyrosine phosphorylation of PLCγ1 and/or PLCγ2 is related causally to the presence and intensity of spontaneous uterine contractions. Specifically, these studies sought to use various inhibitors of the phosphatidylinositol signaling pathway to determine the relationship between PLCγ1 tyrosine phosphorylation and spontaneous uterine contractions.

Materials and Methods

Uterine tissue for these studies was obtained from proestrus/estrus Sprague-Dawley rats (Harlan Sprague Dawley, Inc, Indianapolis, IN) under a protocol approved by the Animal Care and Utilization Committee at the University of Vermont. The in vitro isometric contraction studies were performed at 37°C with the use of longitudinal segments of uterine tissue (6-8 mm relaxed length) in 3-mL muscle baths that contained Earle's balanced salt solution, as previously reported in our laboratory.[7-9] Dose response studies were performed with 1-40 μmol/L potassium bisperoxo (1,10 phenanthroline)oxovanadate [bpV (phen)]) with and without PI-signaling pathway inhibitors: 83 μmol/L phenylarsine oxide (PAO, a selective PLCγ inhibitor in vehicle: dimethylsulfoxide (DMSO), 2.5 mmol/L neomycin (a PLC inhibitor in vehicle: deionized water), 80 μmol/L 2-nitro-4-carboxyphenyl-N,N-

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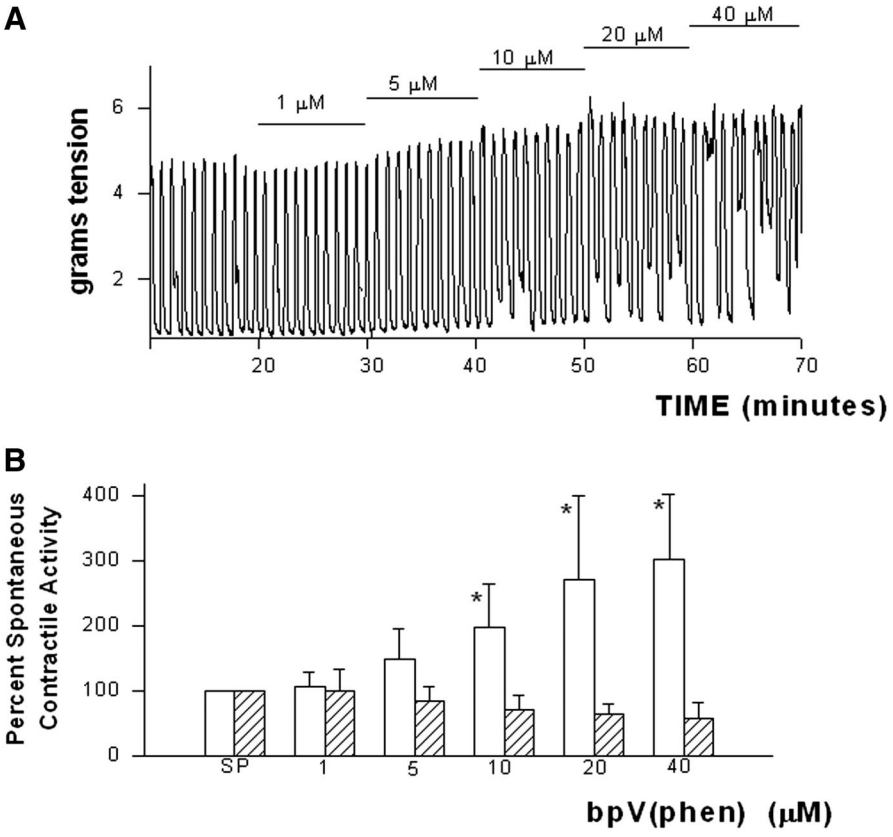
FIGURE 1
BpV(phen)-enhanced spontaneous uterine contractions



A, In vitro contraction study demonstrating the effect of bpV(phen) on spontaneous contractions of the proestrus/estrus rat uterine strips. Cumulative additions of bpV(phen) into the muscle bath for the time periods indicated by the *horizontal bars*. Contractile activity is reported in grams of tension generated. **B,** Bar graph demonstrates the effects of bpV(phen)- (*open bars*) vs vehicle (deionized H_2O)-treated strips (*hatched bars*). Each *bar* denotes the mean ± SD (n = 8 experiments). The *asterisk* denotes a probability value of <.05 for comparison with vehicle-treated strips. *SP*, spontaneous contractile activity preceding the 20-minute time point.

diphenylcarbamate (NCDC, another PLC inhibitor in vehicle [DMSO]), 60 μmol/L 2-aminoethoxydiphenyl borate (2-APB, a calcium entry and inositol triphosphate (IP_3)-receptor inhibitor in vehicle [DMSO]), and 50 μmol/L ruthenium red (a ryanodine receptor inhibitor in vehicle: deionized water). Control studies were performed with comparable volumes of vehicle alone in place of the PI-signaling pathway inhibitors. As previously reported from our laboratory,[7-9] the analog contraction data were computer digitalized, analyzed to determine total contractile area in selected 5-minute intervals, normalized for tissue cross-sectional area, and reported as percent of spontaneous contractile activity (percent control).

For the Western blots, the uterine strips were homogenized in a protease/phosphatase inhibitor 50 mmol Tris buffered saline solution (that contained 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1% Triton X-100, 5 μg/mL aprotinin, 3 μg/mL leupeptin, 1 mmol/L phenylmethylsulfonyl fluoride, and 1 mmol/L sodium orthovanadate). After centrifugation at 800*g* to remove the cellular debris, the protein concentrations of the crude homogenates were determined by bicinchonicic acid (BCA) protein assay (Pierce Biotechnology, Rockville, IL). Subsequently, the proteins were resolved on 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis gels with the Bio-Rad Mini-PROTEAN 3 Electrophoresis System (Bio-Rad Laboratories, Hercules, CA). The resolved proteins were then transferred electrophoretically to nitrocellulose membranes using the Bio-Rad wet transfer system. The nitrocellulose membranes were blocked with 5% powdered milk, then incubated overnight in the primary antibody solution that contained anti-phosphotyrosine PLCγ1 or anti-phosphotyrosine PLCγ2 polyclonal antibodies (Cell Signaling Technology, Danvers, MA) or with anti-PLCγ1 monoclonal or PLCγ2 polyclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) for total PLCγ1 or PLCγ2 protein. Immunodetection of the primary antibodies was performed with horseradish peroxidase-conjugated anti-mouse or rabbit immunoglobulin G antiserum (Bio-Rad Laboratories). Chemiluminescence of the target bands was produced by incubation of the nitrocellulose membranes briefly in Supersignal HRP Substrate (Calbiochem/EMB Biosciences, San Diego, CA), then the nitrocellulose membranes were exposed to Hyperfilm photographic film (Amersham Biosciences, Piscataway, NJ). The relative density of the detected protein bands that were visible on the Hyperfilm was determined with the use of the Kodak 1-D Digital Science Electrophoresis Documentation and Analysis System (Eastman Kodak, Rochester, NY).

Because much of the data was not distributed normally, statistical analyses were performed with the nonparametric Kruskal-Wallis analysis of variance on ranks with multiple comparisons with the Student-Newman-Keuls test or were performed with the Mann-Whitney rank sum test, where appropriate. Statistical significance occurred when the probability value was <.05.

RESULTS

In vitro contraction studies with bpV(phen)

Inhibition of protein tyrosine phosphatase activity using bpV(phen) at concentrations of 1-40 μmol/L produced a marked increase in the contractile activ-