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PKC-MEDIATED REGULATION OF CONTRACTION IS ALTERED IN URINARY BLADDER SMOOTH MUSCLE IN RESPONSE TO PARTIAL BLADDER OUTLET OBSTRUCTION

Hypothesis / aims of study
Partial bladder outlet obstruction (PBOO) is a significant medical problem in men with benign prostatic hyperplasia (BPH). Both in men with BPH and d the rabbit model of PBOO, the detrusor smooth muscle (DSM) undergoes hypertrophy. The aim of the present study was to examine whether PBOO alters the PKC mediated signal transduction pathway (including the expression and activity of PKC and downstream effector of PKC, CPI-17) in bladder smooth muscle contraction.

Study design, materials and methods
PBOO was surgically created in adult male New Zealand White rabbits. Un-operated and sham-operated rabbits served as controls. Two weeks after obstruction, animals were euthanized, bladders were removed and the bladder body muscle layers were cleaned off the adjacent serosal and mucosal tissues. Muscle strips were placed in organ baths containing Tyrode's buffer equilibrated with 95% O_2 - 5% CO_2 at 37ºC and contractile characteristics in response to KCl and carbachol were analyzed. One set of strips were snap-frozen in liquid nitrogen at 30 seconds, 1 minute and 3 minutes respectively after adding 3µM PDBu. A second set of strips were first pre-incubated with 10 µM PKC specific inhibitor, bisindolylmaleimade-1 (Bis) for 20 minutes and then snap-frozen in liquid nitrogen at 30 second, 1 minute and 3 minutes respectively after adding 3µM PDBu. Un-treated strips were used as control. Frozen tissues were saved in liquid nitrogen for subsequent PKC assay and CPI-17 phosphorylation analysis. The expression of CPI-17 was analyzed by real-time PCR and Western blotting at the mRNA and protein levels, respectively

Results
Two weeks after PBOO, the DSM shows altered contractile characteristics. In response to 125 mM KCl or 0.03-3 µM PDBu, the hypertrophied DSM strips from some obstructed bladders generate similar or slightly higher force compared with that of normal, indicating that the hypertrophied DSM is able to contract close to that of normal (compensated). However, the DSM strips from some obstructed bladders show decreased contractility; there is little contraction in response to PDBu and the KCl-induced contraction is ~50% of normal. In these severely dysfunctional bladders (decompensated) with impaired contractility, the expression of PKCα isoform and PKC activity are significantly decreased. Moreover, there is decreased phosphorylation of CPI-17, the downstream effector of PKC-mediated phosphorylation, and this may contribute to the impaired contractility. In contrast, there is no significant difference in the expression of PKC/CPI-17 and PKC activity between normal and obstructed bladders which are compensated. PDBu also induced a significantly higher PKC activity in DSM tissues from normal and compensated bladders compared to that from decompensated bladders. PDBu-induced PKC activity was blocked by the addition of PKC specific inhibitor, Bis. Immunostaining showed that PKC is mainly expressed around membrane and CPI-17 is co-localized with α-smooth muscle actin containing cytoplasmic filaments

Interpretation of results
Our results clearly show that PBOO produces a differential smooth muscle contractile phenotype and differentially alters the PKC pathway with an up-regulation of PKC in compensated bladder smooth muscle and a down-regulation of the PKC pathway in decompensated bladder. In compensated bladder smooth muscle, a high protein expression of PKC contributes to a high enzyme activity which leads to a high level of CPI-17 phosphorylation resulting in a high force generation to PDBu stimulation. In contrast, decompensated bladder smooth muscle generates limited force to PDBu stimulation which is associated with a low PKC activity and expression, leading to a low level of CPI-17 phosphorylation.

Concluding message
Taken together, these findings provide a molecular mechanism for the altered bladder smooth muscle contractility which contributes to decompensated bladder function. In conclusion, our results demonstrate bladder outlet obstruction induces an impaired PKC-mediated contraction bladder smooth muscle which may be due to down-regulation of PKC pathway**.**

Specify source of funding or grant	**George O'Brien Urology Research Grant P50DK052620 from NIH**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**University of Pennsylvania Institutional Animal Care & Use Committee (IACUC).**