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Selective inhibition of neurogenic, but not agonist-induced contractions by phospholipase A_2 inhibitors points to presynaptic phospholipase A_2 functions in contractile neurotransmission to human prostate smooth muscle

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Abstract

Background: Phospholipases A_2 (PLA_2) may be involved in α_1-adrenergic contraction by formation of thromboxane A_2 in different smooth muscle types. However, whether this mechanism occurs with α_1-adrenergic contractions of the prostate, is still unknown. While α_1-adrenoceptor antagonists are the first line option for medical treatment of voiding symptoms in benign prostatic hyperplasia (BPH), improvements are limited, probably by nonadrenergic contractions including thromboxane A_2. Here, we examined effects of PLA_2 inhibitors on contractions of human prostate tissues.

Methods: Prostate tissues were obtained from radical prostatectomy. Contractions were induced by electric field stimulation (EFS) and by α_1-adrenergic agonists in an organ bath, after application of the cytosolic PLA_2 inhibitors ASB14780 and AACOCF3, the secretory PLA_2 inhibitor YM26734, the leukotriene receptor antagonist montelukast, or of solvent to controls.

Results: Frequency-dependent contractions of human prostate tissues induced by EFS were inhibited by 25% at 8 Hz, 38% at 16 Hz and 37% at 32 Hz by ASB14780 (1 μM), and by 32% at 16 Hz and 22% at 32 Hz by AACOCF3 (10 μM). None of both inhibitors affected contractions induced by noradrenaline, phenylephrine or methoxamine. YM26734 (3 μM) and montelukast (0.3 and 1 μM) neither affected EFS-induced contractions, nor contractions by α_1-adrenergic agonists, while all contractions were substantially inhibited by silodosin (100 nM).

Conclusions: Our findings suggest presynaptic PLA_2 functions in prostate smooth muscle contraction, while contractions induced by α_1-adrenergic agonists occur PLA_2-independent. Lacking sensitivity to montelukast excludes

an involvement of PLA_2-derived leukotrienes in promotion of contractile neurotransmission.

KEYWORDS

benign prostatic hyperplasia (BPH), lower urinary tract symptoms (LUTS), phospholipase, prostate smooth muscle contraction, voiding symptoms, α_1-adrenoceptor, α_1-blocker

1 | INTRODUCTION

Voiding symptoms in benign prostatic hyperplasia (BPH) may be caused by urethral obstruction, driven by increased prostate smooth muscle tone and enlargement of the prostate.[1] Contraction of prostate smooth muscle is induced by α_1-adrenoceptors, and by nonadrenergic mediators including thromboxane A_2.[1] α_1-Adrenoceptor antagonists ("α_1-blockers") are the first line option for medical treatment of male voiding symptoms, and commonly believed to improve symptoms by prostate smooth muscle relaxation.[2] However, improvements of symptoms and urinary flow rates are restricted to maximally 50%, contributing to high discontinuation rates, progression, complications, and surgery.[1–3] Limitations of α_1-blockers have been explained by nonadrenergic prostate smooth muscle contractions, induced by thromboxane A_2 and endothelin-1, evidentially capable to raise a maximum prostate smooth muscle tone and being resistant to α_1-blockers.[1,4–6] The limited effectiveness is paralleled by still incomplete understanding of α_1-adrenoceptor functions, in particular at intracellular level, while the interest for nonadrenergic mediators and their relationships to α_1-adrenoceptors is obviously emerging.

In contrast to noradrenaline, released as a neurotransmitter and activating postsynaptic α_1-adrenoceptors, the origin of thromboxane A_2 in the prostate is unknown. Phospholipases A_2 (PLA_2) include cytosolic ($cPLA_2$) and secretory isoforms ($sPLA_2$), producing arachidonic acid from phospholipids, which is further processed to thromboxane A_2 and other eicosanoids.[7,8] In vascular smooth muscle, $cPLA_2$ isoforms are activated by α_1-adrenoceptors, resulting in thromboxane A_2 formation and a thromboxane A_2-mediated component of α_1-adrenergic contraction.[9] Based on findings with picotamide, a dual thromboxane A_2 receptor antagonist and thromboxane synthase inhibitor, inhibiting α_1-adrenergic besides thromboxane-induced smooth muscle contractions in the human prostate, an involvement of similar mechanisms may be supposed for α_1-adrenergic smooth muscle contraction in the prostate.[5,9] However, whether PLA_2 activation takes part in α_1-adrenergic contractions of the prostate as well, is still unknown. Here, we examined effects of different PLA_2 inhibitors on neurogenic and α_1-adrenergic contractions of human prostate tissues, including the $cPLA_2$-selective ASB14780, the $cPLA_2$ inhibitor $AACOCF_3$, and the $sPLA_2$-selective YM26734.

2 | MATERIALS AND METHODS

2.1 | Human prostate tissues

Human prostate tissues were obtained from patients undergoing radical prostatectomy for prostate cancer. Patients with previous transurethral resection of the prostate were excluded. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and has been approved by the ethics committee of the Ludwig-Maximilians University, Munich, Germany (22-0827). Informed consent was obtained from all patients. All samples and data were collected and analyzed anonymously. Accordingly, no patients' data were analyzed or related with sampled tissues. Following removal of prostates from patients, macroscopic examination, and sampling were performed within approximately 30 min by a pathologist. Organ bath studies were started within 3 h following sampling, that is, approximately 3.5 h following surgical removal. For transport and storage, prostates and tissues were stored in Custodiol® solution (Köhler). For macroscopic examination and sampling, the prostate was opened by a single longitudinal cut from the capsule to the urethra. Subsequently, both intersections were checked macroscopically for any obvious tumor infiltration. Tissues were taken solely from the transitional, periurethral zone, as most prostate cancers arise in the peripheral zone. In fact, tumor infiltration in the periurethral zone occurred in less than 1% of examined prostates. Prostates showing tumors in the periurethral zone upon macroscopic inspection were not included in this study.

2.2 | Organ bath

Prostate strips ($6 \times 3 \times 3$ mm) were mounted in organ baths (Danish Myotechnology) with four chambers, stretched to 4.9 mN, equilibrated, and contracted by 80 mM KCl as previously described.[10] Following washout

of KCl, inhibitors or solvent (dimethylsulfoxide, DMSO, or ethanol) for controls were added. Cumulative concentration response curves for α_1-adrenergic agonists or frequency response curves for electric field stimulation (EFS) were constructed 30 min after addition of inhibitors or solvent. EFS induces neurogenic contractions, and was applied as previously described.[10]

Each independent experiment was performed using tissue from the same prostate, which was examined with inhibitor and as control group. Only one concentration response or frequence response curve was recorded with each sample. Wherever possible, double determinations were performed. For double determinations, two of the four organ bath channels were examined with inhibitor, and the two others with solvent. From a total of 124 experiments, double determinations in both groups were possible in 101 experiments. In the remaining experiments, the amount of sampled tissues did not allow filling of two channels for both groups, so that single determinations were performed in one group, or rarely in both groups. However, each experiment contained at least one sample for both groups, resulting in paired samples. Allocations of channels to control and inhibitor groups were changed between experiments.

Agonist- and EFS-induced contractions are expressed as percentage of 80 mM KCl-induced contractions, as this may correct varying phenotypes and degrees of BPH, or any individual variation and heterogeneity between samples and patients. E_{max} values, EC_{50} values for agonists, and frequencies (f) inducing 50% of the maximum EFS-induced contraction (Ef_{50}) were calculated separately for each single experiment by curve fitting using GraphPad Prism 6 (GraphPad Software Inc). Concentration and frequence response curves were fitted by nonlinear regression (three parameters), without predefined constraints for bottom, top or EC_{50} values, by ordinary fit, without weighting, and without chosing automatic outlier elimination. While analysis of EFS data by nonlinear regression is technically possible, the generated data may be of limited conclusiveness, for example, owing to the low or lacking sigmoidal character of frequence response curves. Taking any limitation into account, E_{max} and Ef_{50} values reported for EFS experiments need to be considered as an approximation. Error messages, send by the program if curve fitting is not possible, or if results from curve fitting are suspected as "ambiguous" or nonplausible did not occur. In addition and as recommended in the "GraphPad Curve Fitting Guide" (GraphPad Software Inc), values from curve fitting were checked manually for plausibility, resulting in omission of one experiment (methoxamine with/without YM26734) in scatter plots for E_{max}/EC_{50} values, but not from statistical analyses or in the corresponding concentration response curve.

2.3 | Materials, drugs, and nomenclature

ASB14780 (3-(3-Phenethyl-1-(4-phenoxyphenyl)-1H-indol-5-yl)propanoic acid tris salt) is a $cPLA_{2\alpha}$ inhibitor, inhibiting arachidonic acid formation with an IC_{50} of 20 nM in biochemical assays using purified $cPLA_{2\alpha}$, and with an IC_{50} of 500 nM by ASB14780 in a cell-based assay using human monoblast U937 cells.[11] Formation of thromboxane B_2 in whole blood assays was inhibited with an IC_{50} of 640 nM using human blood, and 540 nM using guinea-pig blood.[11] No inhibition of purified $sPLA_2$ was observed using 10 μM ASB14780 in biochemical assays, suggesting selectivity for $cPLA_2\alpha$ over $sPLA_2$.[11] $AACOCF_3$ (1,1,1-Trifluoro-6*Z*,9*Z*,12*Z*,15*Z*-heneicosateraen-2-one) is a $cPLA_2$ inhibitor, inhibiting arachidonic acid formation with an IC_{50} of 8.5 μM in biochemical assays using purified $cPLA_{2\alpha}$, and completely with 30 μM.[12] In human platelets, arachidonic acid formation was inhibited by $AACOCF_3$ with an IC_{50} of 2 μM, and completely using 15 μM.[12] Consequently, $AACOCF_3$ inhibited the thromboxane B_2 formation by 70%–90%, and increased formation of the eicosanoid 12-HETE by 2.5-3 fold in human platelets, using 15 μM.[12] In addition to $cPLA_2$, $AACOCF_3$ inhibits fatty acid amide hydrolase (FAAH), with an IC_{50} of 6 μM or to 100% with 7.5 μM, depending on conditions in biochemical assays, and elevating anandamide levels in intact neuroblastoma cells 12 fold using 12 μM.[13] YM26734 (1,1′-[5-[3,4-Dihydro-7-hydroxy-2-(4-hydroxyphenyl)-2H-1-benzopyran-4-yl]-2,4,6-trihydroxy-1,3-phenylene]bis-1-dodecanone) is a competitive $sPLA_2$ inhibitor, inhibiting several $sPLA_2$ isoforms with IC_{50} values ranging from 0.2 to 3 μM in biochemical assays using recombinant phospholipases.[14] Purified $cPLA_2$ was not inhibited using 50 μM in biochemical assays.[15] Montelukast (1-[[[(1R)-1-[3-[(1E)-2-(7-Chloro-2-quinolinyl)ethenyl]phenyl]-3-[2-(1-hydroxy-1-methylethyl)phenyl]propyl]thio]methyl]cyclopropaneacetic acid) is a cysteinyl leukotriene receptor 1 (CysLT1 receptor) antagonist, where it binds with a K_i of 2.5 nM.[16] Silodosin (1-(3-hydroxypropyl)-5-[(2R)-2-[2-[2-(2,2,2-trifluoroethoxy)phenoxy]ethylamino]propyl]-2,3-dihydroindole-7-carboxamide) is an α_1-adrenoceptor antagonist being available for treatment of male voiding symptoms,[2] showing affinities of 32 pM for α_{1A}, 6.4–19 nM for α_{1B} and 1.6–1.8 nM for α_{1D} in binding studies with recombinant human α_1-adrenoceptors.[17,18] Noradrenaline-induced contractions of human prostate tissues were antagonized with an affinity of 355 pM.[19] Stock solutions (10 mM) of ASB14780 and montelukast were prepared with DMSO. Stock solutions (10 mM) of $AACOCF_3$ and YM26734 were prepared with ethanol. Stock solutions (10 mM) of silodosin were prepared with ethanol, and diluted to 100 μM. Aliquots were stored at −20°C until

use. Phenylephrine ((R)-3-[-1-hydroxy-2-(methylamino) ethyl]phenol) and methoxamine (α-(1-Aminoethyl)-2,5-dimethoxybenzyl alcohol) are α_1-selective adrenoceptor agonists. Aqueous stock solutions (10 mM) of noradrenaline, phenylephrine, and methoxamine were freshly prepared before each experiment. ASB14780, $AACOCF_3$, YM267334, montelukast and silodosin were obtained from Tocris. Noradrenaline, phenylephrine, and methoxamine were obtained from Sigma-Aldrich.

2.4 | Data and statistical analyses

Data in concentration and frequency response curves are means ± standard deviation (SD). E_{max}, EC_{50} and Ef_{50} values are presented as single values (means from double determination, where this was possible) together with means from all independent experiments in scatter plots. In the text, effect sizes are reported as mean differences (MD) with 95% confidence intervals (CIs) for E_{max} values and using original units, or as inhibitions at single frequencies and agonist concentrations, calculated by normalization of values with inhibitor to the corresponding controls in each single experiment, and expressed as means with 95% CIs. Calculation of MDs and 95% CIs, and statistical analyses were performed using GraphPad Prism 6. Comparison of whole curves was performed by two-way analysis of variance (ANOVA), without multiple comparison. E_{max}, EC_{50} and Ef_{50} values were compared by a paired Student's t test. $p < 0.05$ were considered significant. The present study and analyses show an exploratory design, and were not designed to test prespecified statistical null hypotheses. Consequently, p values reported here need to be considered as descriptive, but not as hypothesis-testing.[20] Minimum group sizes were preplanned as $n = 5$ for each series, to allow calculation of descriptive p values. Thus, series were discontinued after five independent experiments, if it was obvious that no effect could be expected, or if $p < 0.05$ was observed between both groups in frequency/concentration response curves. Results were inconclusive after five initial experiments in one series, which was continued and finally analyzed after three further experiments.

3 | RESULTS

3.1 | Effects of ASB14780

EFS (2–32 Hz) induced frequence-dependent contractions of human prostate tissues, which were reduced with 1 μM ASB14780, compared to DMSO-treated controls (Figure 1A). Decreases in contractions amounted to 25% [−16 to 66] at 8 Hz, 38% [18–59] at 16 Hz and 37% [16–58] at 32 Hz. E_{max} values were decreased in each experiment, from 191% [150–231] of KCl-induced contractions in controls to 121% [84–159] of KCl-induced contractions with ASB14780 (MD 70 KCl percentage points [22–118]) (Figure 1A). Ef_{50} values were not changed (Figure 1A).

Noradrenaline, phenylephrine, and methoxamine (0.1–100 μM) induced concentration-dependent contractions, which were not reduced (phenylephrine) or to neglectable degree (noradrenaline, methoxamine) by 1 μM ASB14780 (Figure 1B–D). No consistent effects were seen in concentration response curves, on E_{max} values or on EC_{50} values for α_1-adrenerigc agonists (Figure 1B–D).

3.2 | Effects of $AACOCF_3$

EFS-induced contractions were reduced with 10 μM $AACOCF_3$, compared to ethanol-treated controls (Figure 2A). Decreases in contractions amounted to 32% [−9 to 73] at 16 Hz and 22% [−22 to 66] at 32 Hz (Figure 2A). E_{max} values were decreased in four of a total of five independent experiments, from 113% [14–213] of KCl-induced contractions in controls to 71% [19–123] of KCl-induced contractions with $AACOCF_3$ (MD 42 KCl percentage points [23–106]) (Figure 2A). Ef_{50} values were not substantially changed (Figure 2A).

Contractions by noradrenaline, phenylephrine and methoxamine were not reduced by 10 μM $AACOCF_3$ (Figure 2B–D). No inhibitions were seen in concentration response curves or for E_{max} values, and no consistent effects were seen on EC_{50} values for α_1-adrenerigc agonists (Figure 2B–D). Slight increases of contractions seen with $AACOCF_3$ and for all three agonists were neglectable for noradrenaline and phenylephrine, or were caused by one outlier experiment for methoxamine (Figure 2D).

3.3 | Effects of YM26734

EFS-induced contractions were not changed by 3 μM YM26734 (Figure 3A). An increase on average Ef_{50} values was in fact caused by increased values in two out of a total of five independent experiments.

Contractions by noradrenaline, phenylephrine and methoxamine were neither substantially, nor consistently changed (Figure 3B–D). Possible decreases by YM26734 were seen in concentration response curves for all three agonists, but were neglectable for noradrenaline (Figure 3B), or characterized by high variation and

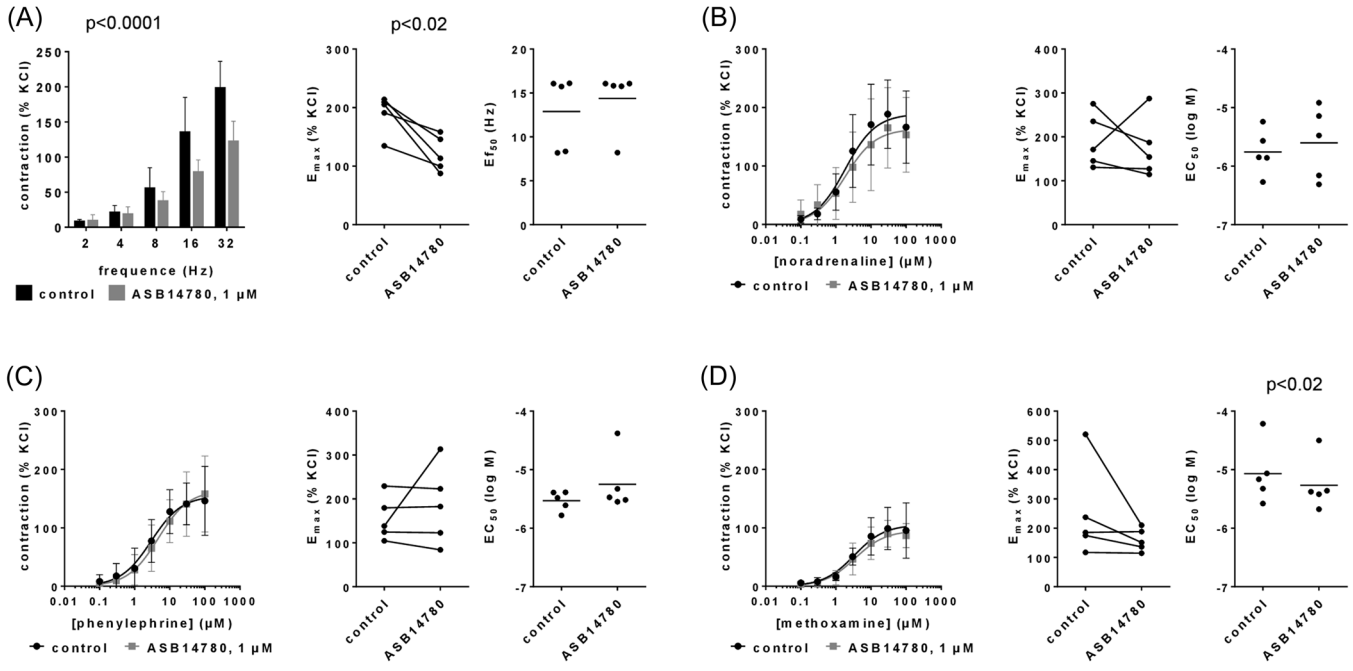

FIGURE 1 Effects of ASB14780 on EFS-induced and adrenergic contractions of human prostate tissues. Contractions of human prostate tissues were induced by EFS (A), noradrenaline (B), phenylephrine (C), or methoxamine (D) in an organ bath, 30 min after addition of ASB14780 (1 µM) or of an equivalent amount of DMSO (controls), which was used as solvent for ASB14780. Shown are data from $n = 5$ independent experiments in each panel, where tissues from $n = 5$ patients were used for both groups of a subpanel, resulting in paired samples. Shown are means ± SD from all experiments in frequence and concentration response curves with p values from two-way ANOVA for whole groups, and all single E_{max}, EC_{50}, and Ef_{50} values from all experiments (calculated by curve fitting, with corresponding E_{max} values obtained from the same tissues connected with each other) together with p values from Student's t test in scatter plots. ANOVA, analysis of variance; EFS, electric field stimulation.

limited to the highest agonist concentration for phenylephrine and methoxamine (Figure 3C,D). A decreased average E_{max} value for phenylephrine was caused by one outlier experiment, while E_{max} values for noradrenaline or methoxamine were unchanged, and EC_{50} values were not substantially changed (Figure 3B–D).

3.4 | Effects of montelukast

EFS-induced contractions, and contractions by noradrenaline, phenylephrine and methoxamine were not changed by montelukast, using concentrations of 0.3 or 1 µM (Figure 4). A slight increase in phenylephrine- and methoxamine-induced contractions with 0.3 µM montelukast was neither consistent with unchanged contractions by noradrenaline, nor with unchanged contractions after application of 1 µM montelukast (Figure 4).

3.5 | Effects of silodosin

To validate that limited or lacking effects of PLA_2 inhibitors were not due to technical artefacts, inhibition of EFS-induced and α_1-adrenergic contractions was reproduced using silodosin (Figure 5). Silodosin (100 nM) caused profound inhibitions of EFS-, noradrenaline-, phenylephrine-, and methoxamine-induced contractions, as seen in frequence and concentration response curves (Figure 5). Calculation of conclusive E_{max} and EC_{50} values for α_1-adrenergic agonists was not possible, as contractions did not recover with silodosin within the applied ranges of agonist concentrations.

4 | DISCUSSION

Our findings obtained with ASB14780 and AACOCF3 may suggest a $cPLA_2$-dependent mechanism involved in contractile neurotransmission in the prostate, as both compounds inhibited EFS-induced contractions, but no contractions by α_1-adrenergic agonists. Our study was initiated by the previous observation that picotamide, a dual thromboxane A_2 receptor antagonist and thromboxane synthase inhibitor inhibited α_1-adrenerigc, in addition to thromboxane-induced contractions in prostate and vascular smooth muscle.[5,6,9] Consequently, we supposed that activation of PLA_2 by α_1-adrenoceptors,

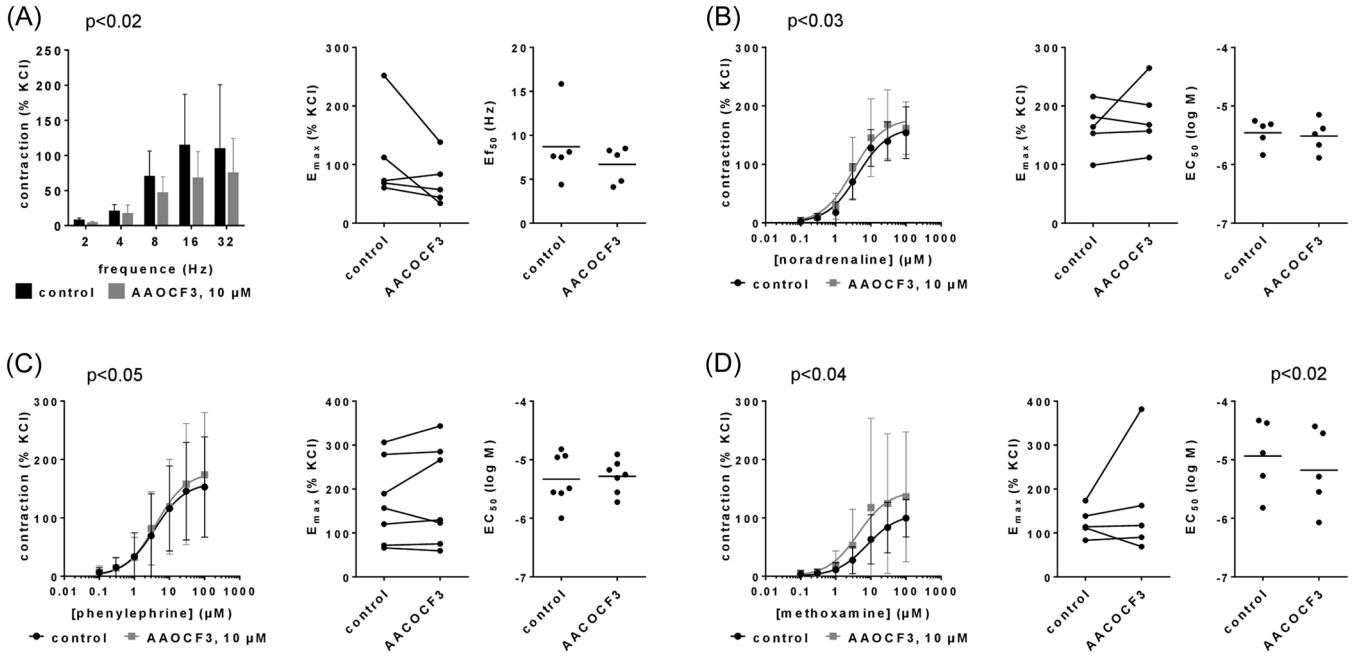

FIGURE 2 Effects of $AACOCF_3$ on EFS-induced and adrenergic contractions of human prostate tissues. Contractions of human prostate tissues were induced by EFS (A), noradrenaline (B), phenylephrine (C), or methoxamine (D) in an organ bath, 30 min after addition of $AACOCF_3$ (10 µM) or of an equivalent amount of ethanol (controls), which was used as solvent for $AACOCF_3$. Shown are data from $n = 5$ independent experiments in (A), (B), and (D), and from $n = 7$ independent experiments in (C), where tissues from $n = 5$ or $n = 7$ patients were used for both groups of a subpanel, resulting in paired samples. Shown are means ± SD from all experiments in frequence and concentration response curves with p values from two-way ANOVA for whole groups, and all single E_{max}, EC_{50}, and Ef_{50} values from all experiments (calculated by curve fitting, with corresponding E_{max} values obtained from the same tissues connected with each other) together with p values from Student's t test in scatter plots. ANOVA, analysis of variance; EFS, electric field stimulation.

followed by production of thromboxane A_2 could be involved in α_1-adrenerigc contractions of human prostate tissues.[9] In fact, a thromboxane A_2-mediated component of α_1-adrenergic contractions has been repeatedly proposed for vascular smooth muscle, while data describing effects of PLA_2 inhibitors on prostate smooth muscle contraction were to the best of our knowledge not available.

ASB14780 and $AACOCF_3$ are structurally unrelated inhibitors and shared off-targets have not been reported, supporting the conception that the inhibition of EFS-induced contractions was imparted by $cPLA_2$ inhibition. Neurogenic contractions in the human prostate are caused by adrenergic neurotransmission, followed by activation of postsynaptic α_{1A}-adrenoceptors, while details of a possible $cPLA_2$-mediated promotion remain speculation at this stage. Action potentials resulting in release of catecholamines, and activation of $cPLA_2$ isoforms are both calcium-dependent.[7] Thus, it appears possible that $cPLA_2$ isoforms are involved in transport of synaptic vesicles to synapses, in fusion of vesicles with membranes, or in exocytosis of catecholamines. Alternatively, neurogenic contractions depending on $cPLA_2$ may be caused by release of eicosanoids, including arachidonic acid or leukotrienes, the latter promoting smooth muscle contractions in the cardiovascular system and airways.[21,22] Data supporting analog roles of leukotrienes or arachidonic acid in prostate smooth muscle contraction are not yet available, while our current findings with montelukast may exclude a release of leukotrienes as a contractile neurotransmitter. Arachidonic acid, in turn, activates Rho kinase, which is a prototypical intracellular mediator in smooth muscle contraction.[23–25] A release of thromboxane A_2 during neurotransmission can be excluded in our experiments, as EFS-induced contractions of human prostate tissues were resistant to the thromboxane A_2 receptor antagonists in a previous study.[5]

PLA_2 activation by contractile agonists and subsequent formation of arachidonic acid or thromboxane A_2 have been reported from different smooth muscle types. In vascular smooth muscle cells, α_1-adrenoceptors, angiotensin-II and serotonin activate PLA_2 and cause arachidonic acid formation, which may contribute to agonist-induced vasocontraction.[26–30] Similar concepts were suggested by findings with nonadrenergic mediators, and for airway, esophageal, glomerular, and sphincter smooth muscle.[9] Consequently, we speculated

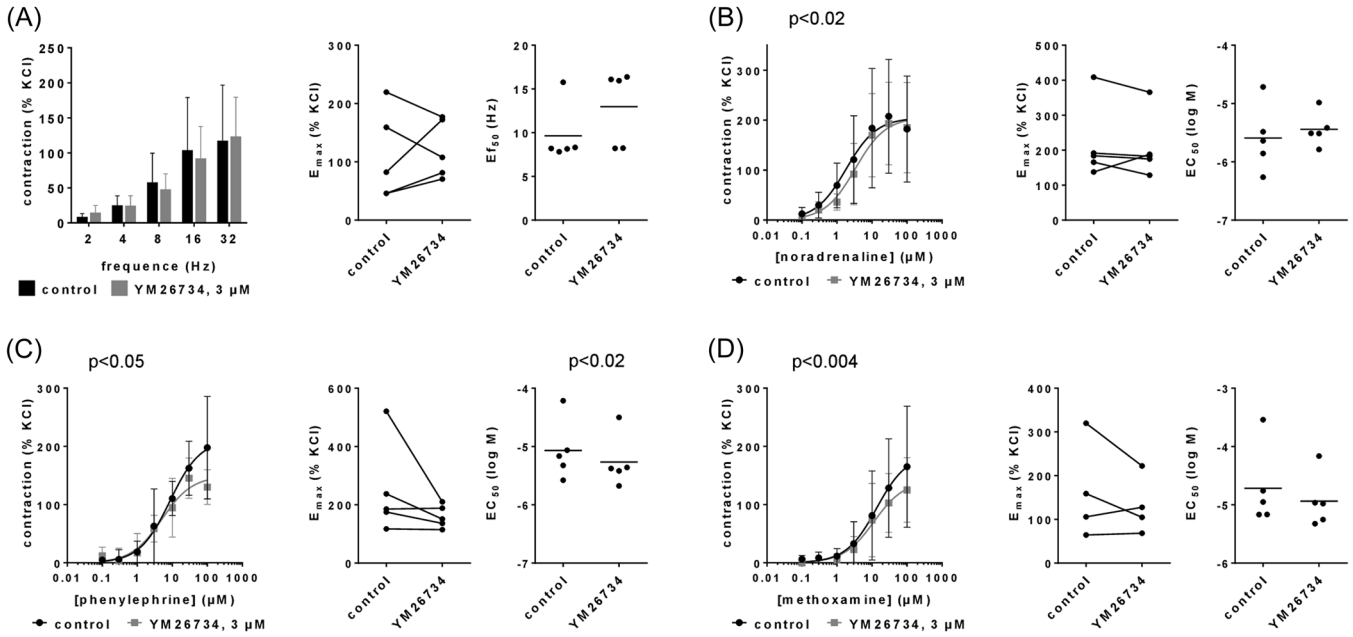

FIGURE 3 Effects of YM26734 on EFS-induced and adrenergic contractions of human prostate tissues. Contractions of human prostate tissues were induced by EFS (A), noradrenaline (B), phenylephrine (C), or methoxamine (D) in an organ bath, 30 min after addition of YM26734 (3 µM) or of an equivalent amount of ethanol (controls), which was used as solvent for YM26734. Shown are data from $n = 5$ independent experiments in each panel, where tissues from $n = 5$ patients were used for both groups of a subpanel, resulting in paired samples. Shown are means ± SD from all experiments in concentration response curves with p values from two-way ANOVA for whole groups, and all single E_{max}, EC_{50}, and Ef_{50} values from all experiments (calculated by curve fitting, with corresponding E_{max} values obtained from the same tissues connected with each other) together with p values from Student's t test in scatter plots. ANOVA, analysis of variance; EFS, electric field stimulation.

that activation of $cPLA_2$ by α_1-adrenoceptors and subsequent $cPLA_2$-mediated thromboxane A_2 formation contributes to α_1-adrenergic prostate smooth muscle contractions. However, our findings do not provide a basis to assume, that this mechanism of α_1-adrenergic contraction is shared by prostate smooth muscle.

Previous studies addressing PLA_2 functions in the nonmalignant prostate did not include smooth muscle contraction. PLA_2 purified from bovine prostates was calcium-dependent, suggesting that the predominant isoforms are cytosolic.[31] Similar conclusions may be drawn from our findings with human tissues, as no effects occurred with an inhibitor for secretory isoforms. In biochemical assays with homogenates from stromal and epithelial cells obtained from patients with BPH, addition of endogenous phospholipase caused a 5α-reductase activity, suggesting that phospholipid composition in the prostate may be decisive for androgen-dependent growth in BPH.[32] Consequently, it appears possible that $cPLA_2$ isoforms play a dual role, in prostate growth and neurogenic prostate smooth muscle contraction, both believed to drive voiding symptoms in BPH. However, the role for prostate smooth muscle tone appears limited, as inhibition of EFS-induced contraction by $cPLA_2$ inhibitors amounted to less than 50% in our experiments.

To exclude that lacking inhibitions of agonist-induced contractions by PLA_2 inhibitors were attributed to technical artefacts in our hands, we reproduced inhibitions of α_1-adrenergic and EFS-induced contractions by silodosin. The lacking recovery of α_1-adrenergic contractions in the presence of silodosin may be attributed to the limited range of applied concentrations, while a noncompetitive component of α_1-blockers in smooth muscle contractions has been in fact previously supposed. The concentrations of PLA_2 inhibitors applied in our experiments were fully in the range causing PLA_2 inhibition in previous studies. At least for $AACOCF_3$, additional inhibition of FAAH can not be excluded, which may result in accumulation of the endocannabinoid anandamide. Inhibitory effects of cannabinoid agonists have been reported for neurogenic prostate smooth muscle contractions, but are apparently lacking for contractions induced by α_1-adrenergic agonists,[33,34] resembling to the pattern of inhibition observed in our current study.

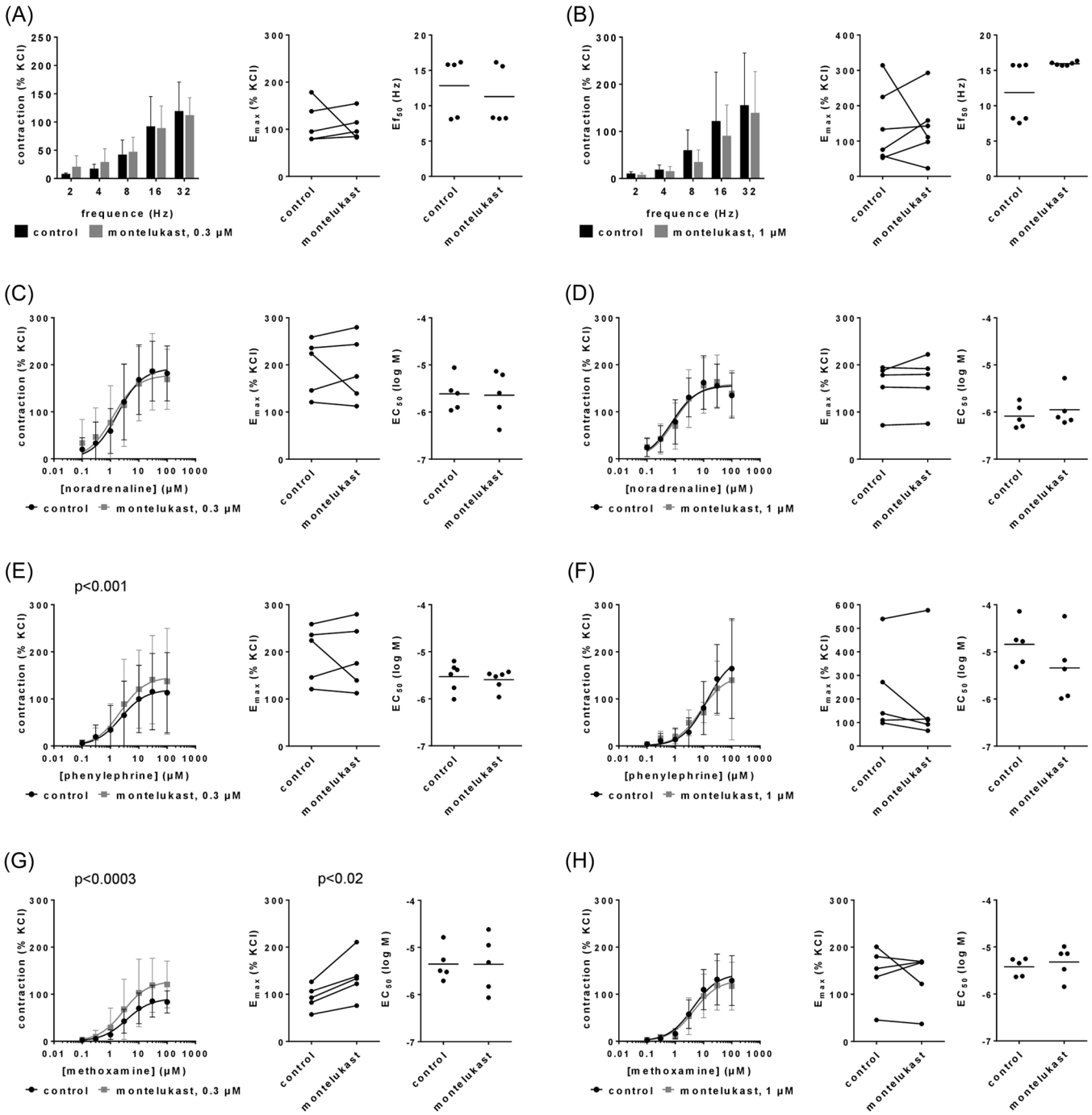

FIGURE 4 Effects of montelukast on EFS-induced and adrenergic contractions of human prostate tissues. Contractions of human prostate tissues were induced by EFS (A, B), noradrenaline (C, D), phenylephrine (E, F), or methoxamine (G, H) in an organ bath, 30 min after addition of YM26734 (0.3 or 1 µM, as indicated) or of an equivalent amount of DMSO (controls), which was used as solvent for montelukast. Shown are data from $n = 6$ independent experiments in (B) and (E), and from $n = 5$ patients in all other panels, where tissues from $n = 5$–6 patients were used for both groups of a subpanel, resulting in paired samples. Shown are means ± SD from all experiments in concentration response curves with p values from two-way ANOVA for whole groups, and all single E_{max}, EC_{50}, and Ef_{50} values from all experiments (calculated by curve fitting, with corresponding E_{max} values obtained from the same tissues connected with each other). ANOVA, analysis of variance; EFS, electric field stimulation.

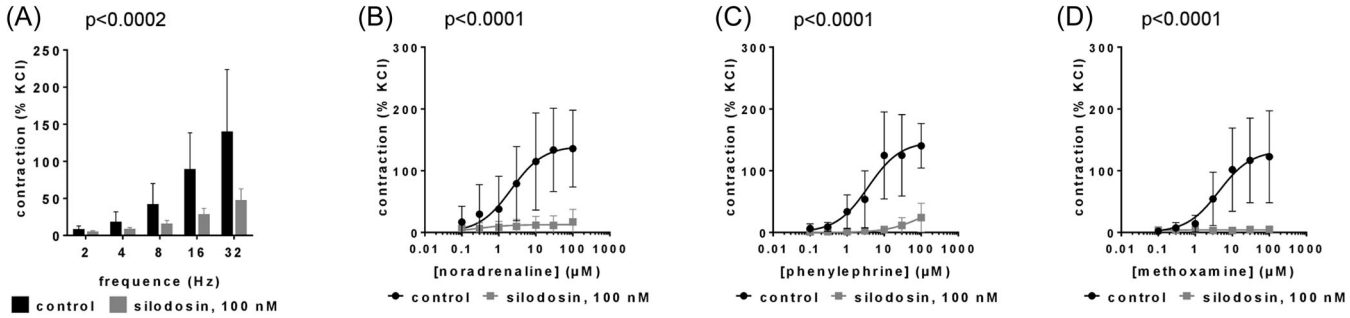

FIGURE 5 Effects of silodosin on EFS-induced and adrenergic contractions of human prostate tissues. Contractions of human prostate tissues were induced by EFS (A), noradrenaline (B), phenylephrine (C), or methoxamine (D) in an organ bath, 30 min after addition of silodosin (100 nM) or of an equivalent amount of ethanol (controls), which was used as solvent for silodosin. Shown are data from $n = 5$ independent experiments in each panel, where tissues from $n = 5$ patients were used for both groups of a subpanel, resulting in paired samples. Shown are means ± SD from all experiments with p values from two-way ANOVA for whole groups. ANOVA, analysis of variance; EFS, electric field stimulation.

5 | CONCLUSIONS

Selective inhibition of neurogenic, but not α_1-adrenergic contractions by cPLA$_2$ inhibitors suggests presynaptic PLA$_2$ functions in prostate smooth muscle contraction, while contractions induced by α_1-adrenergic agonists occur PLA$_2$-independent. Lacking sensitivity to montelukast excludes an involvement of PLA$_2$-derived leukotrienes in promotion of contractile neurotransmission.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data sets generated for this study and supporting the findings of this study are included in the manuscript. The raw data of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study was performed with prostate tissues obtained from radical prostatectomy for prostate cancer, which were used for in vitro experiments. All tissues and data were collected and analyzed anonymously. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and has been approved by the ethics committee of the Ludwig-Maximilians University, Munich, Germany (22-0827). The manuscript does not contain material from other sources. Informed consent was obtained from all patients.

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