

Picotamide inhibits a wide spectrum of agonist-induced smooth muscle contractions in porcine renal interlobar and coronary arteries

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Abstract

Picotamide is a thromboxane A₂ (TXA₂) receptor antagonist and TXA₂ synthase inhibitor. In clinical studies, it has been considered as a platelet aggregation inhibitor and improved renal function. In vitro studies suggested inhibition of smooth muscle contraction by picotamide, which is poorly understood. Here, we examined effects of picotamide on contractions of renal interlobar and coronary porcine arteries, induced by different vasoconstrictors. Contractions were induced in an organ bath by agonists or electric field stimulation (EFS). Picotamide inhibited EFS-induced contractions of interlobar arteries around 50% using concentrations of 100 and 300 μM. In interlobar arteries, concentration response curves for contractions induced by three different α₁-adrenoceptor agonists were shifted to the right by picotamide (2–10-fold increases in EC₅₀). In coronary arteries, α₁-adrenergic contractions were inhibited without right shift (approx. 50%). Contractions induced by two different cholinergic agonists in coronary arteries were inhibited by picotamide (≥50%) without right shift. Inhibition of serotonin-induced contractions by picotamide showed features of a right shift, whereas contractions induced by the TXA₂ analog U46619, angiotensin-II, and endothelin-1 were inhibited by picotamide in interlobar and coronary arteries without right shifts and to different degree. Picotamide inhibits a wide spectrum of vasoconstrictor-induced contractions in porcine interlobar and coronary arteries. Inhibition of vasoconstriction may contribute to beneficial effects of picotamide in the cardiovascular system and kidney.

KEYWORDS

organ bath, picotamide, smooth muscle contraction, vascular smooth muscle, vasoconstrictor

Abbreviations: DMSO, dimethylsulfoxide; EFS, electric field stimulation; TXA₂, thromboxane A₂.

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1 | INTRODUCTION

Picotamide is known as a combined thromboxane A_2 receptor antagonist and thromboxane synthase inhibitor, which inhibits thromboxane A_2 -induced platelet aggregation.^{1,2} It has been tested as an antiplatelet drug in clinical trials, resulting in approval as a platelet aggregation inhibitor in Italy.^{3–8} Recent meta-analyses have considered applications in prevention and prophylaxis of cardiovascular disease.^{9,10} However, clinical and preclinical studies suggested that effects of picotamide are not limited to antiplatelet activity, or to inhibition of thromboxane A_2 -mediated functions.

In patients with type 2 diabetes, picotamide improved renal function and increased the intrarenal vascular resistance and perfusion.³ Although inhibition of intrarenal vasoconstriction rather than platelet aggregation was proposed to account for beneficial effects of picotamide on kidney function, this has never been fully explained. Preclinical studies pointed to thromboxane A_2 receptor-independent effects, in particular in smooth muscle contraction. Thus, besides platelet aggregation, picotamide inhibited vascular, prostate, and trigone smooth muscle contractions *in vitro*.^{11–13} In addition to an expectable, competitive inhibition of thromboxane A_2 receptor-induced contractions, inhibition of α_1 -adrenoceptor- and serotonin-induced contractions with non-competitive features was observed in rabbit aortic rings.¹³ In rat aortic rings, picotamide inhibited acetylcholine-induced contractions.¹⁴ Similarly, effects on contractions on other agonists than thromboxane A_2 have been reported from lower urinary tract smooth muscle.^{11,12} Application of picotamide to human tissues suggested inhibition of α_1 -adrenergic, neurogenic and endothelin-induced smooth contractions in the prostate, and of cholinergic, α_1 -adrenergic and neurogenic smooth muscle contractions in the bladder trigone, besides inhibition of thromboxane A_2 -induced contractions.^{11,12}

Together, properties and actions of picotamide are obviously still incompletely understood, despite its applications in clinical studies and as an antiplatelet drug. Acknowledging the importance of smooth muscle contraction for cardiovascular homeostasis and diseases, but also considering potential applications in treatment of lower urinary tract symptoms, where cardiovascular side effects may be critical,¹⁵ adequate understanding of vascular picotamide effects may be clinically relevant. Evidence for actions of picotamide on vascular smooth muscle contractions other than thromboxane A_2 induced is to the best of our knowledge limited to phenylephrine-, serotonin-, and acetylcholine-induced contractions in rabbit and rat aortic rings,^{13,14} and did not include other vessel types, other species, or neurogenic contractions. Apart from thromboxane A_2 , α_1 -adrenoceptors, and serotonin, the spectrum of vasoconstrictors inducing receptor-dependent contractions of vascular smooth muscle includes angiotensin-II and endothelin-1, besides others. To obtain a broader understanding of vascular picotamide effects, we here examined the effects of picotamide on neurogenic and agonist-induced vascular smooth muscle contractions of porcine interlobar kidney and coronary arteries.

2 | MATERIALS AND METHODS

2.1 | Pig arteries

Kidneys and hearts from pigs were obtained from a local slaughterhouse, where animals were killed during night. Subsequently, organs were picked up by a local butcher, transported and stored at 4°C, and transferred from the butcher's shop (Metzgerei Brehm, Planegg, Germany) to the laboratory in the morning. Here, interlobar arteries were prepared from kidneys, and middle sections of left anterior descending arteries were prepared from hearts. Preparation was started approximately 15 min following pickup of organs from the butcher. Following preparation and dissection of adipose and connective tissues, vessels were cut into rings, transferred to Custodiol® solution (Köhler, Bensheim, Germany), and stored at 4°C in Custodiol solution until use. Experiments were started not later than three hours following preparation of vessels.

2.2 | Organ bath studies

Vessel rings (i.e., segments cutted from vessels) were mounted in tissue baths with four chambers (Danish Myotechnology, Aarhus, Denmark). Rings had a length of 2–3 mm, with diameters ranged between 3 and 4 mm for renal interlobar arteries, and around 5 mm for coronary arteries. Each chamber contained 10 ml aerated (95% O_2 and 5% CO_2) Krebs–Henseleit solution (37°C, pH 7.4). For pre-tension, rings of interlobar arteries were stretched to 9.8 mN, whereas rings of left anterior descending arteries were stretched to 19.6 mN. In the initial phase of the equilibration period, spontaneous decreases in tone are usually observed. Therefore, tension was adjusted three times during the equilibration period, until a stable resting tone of 9.8 or 19.6 mN, respectively, was attained. After the equilibration period, maximum contraction induced by 80 mM KCl was assessed. As soon as a plateau contraction induced by KCl was obtained, chambers were washed three times with Krebs–Henseleit solution for a total of 30 min, and picotamide or an equivalent amount of dimethylsulfoxid (DMSO, used as solvent for picotamide) was added for controls. Cumulative concentration response curves for agonists, or frequency response curves for electric field stimulation (EFS), were constructed 30 min after addition of picotamide or DMSO. Application of EFS simulates action potentials, resulting in the release of endogenous neurotransmitters. Picotamide was examined in final concentrations of 30, 100, and 300 μ M in experiments with EFS-induced contractions, and 300 μ M in experiments with agonist-induced contractions. A stock solution of 300 mM was applied to organ bath chambers, so that final concentrations of DMSO amounted to 0.1‰, 0.33‰, and 1‰ for in EFS experiments, and 1‰ for in agonist experiments.

In each experiment, all four chambers of one organ bath were filled with rings from the same vessel, and only one concentration or frequency response curve was recorded with each chamber. From these four channels, two were examined with picotamide,

and two with DMSO, with changing allocations of picotamide and DMSO channels between different experiments. Each of these independent experiments was repeated in indicated numbers (n), with arteries from n different animals, resulting indicated numbers of experiments in each series. Consequently, control and picotamide groups in each series (i.e., each diagram in figures) were obtained using the same vessels, and single experiments were based on double determinations.

For calculation of agonist- and EFS-induced contractions, tensions were expressed as percentage of 80 mM KCl-induced contractions, to correct for inter-strip variabilities. In fact, normalization to KCl allows to examine possible alterations of receptor responsiveness, whereas correlations between agonist-induced force and ring weight, length, or cross-sectional area are weak or lacking in organ bath experiments using vessel segments.¹⁶

E_{\max} values, EC_{50} values for contractile agonists, and frequencies (f) inducing 50% of the maximum EFS-induced contraction (E_{f50}) were calculated by curve fitting using GraphPad Prism 6 (Statcon, Witzenhausen, Germany), and analyzed as described below. As presentation of single values in scatter plots was intended, automatic curve fitting was performed separately for each single experiment, to obtain single values for each independent experiment. Sigmoidal concentration response curves were fitted by non-linear regression, without predefined constraints for bottom, top or EC_{50} values, by ordinary fit, without weighting, and without choosing automatic outlier elimination. However, and as recommended in the "GraphPad Curve Fitting Guide" (GraphPad Software Inc., San Diego, CA, USA), resulting values were checked for plausibility, and settings were adapted as follows if error messages occurred. Thus, curve fitting of concentration response curves for serotonin was performed using sigmoidal parts of curves, i.e. including the concentration range from 0.1 to 100 μ M (0.1 to 10 μ M in one case), but excluding steady-state or downhill portions at high concentrations, as fitting with all concentrations (up to 1 mM) resulted in error messages („not converted“) in 3 out of 6 experiments. Similarly, the concentration range from 30 to 100 μ M was excluded in one carbachol experiment, as maximum contractions occurred at low concentrations in this experiment, resulting in otherwise non-plausible results. Results from curve fitting were flagged as "ambiguous" in one methoxamine experiment in renal arteries, so that curve fitting was performed using the concentration range of 0.1–10 μ M. Finally, and despite these procedures, one outlier occurred, which was handled as described in Section 2.4.

2.3 | Materials, drugs, and nomenclature

Picotamide [4-methoxy-N,N'-bis(pyridin-3-ylmethyl)isophthalamide] is a combined antagonist of the thromboxane A_2 receptor and inhibitor of thromboxane A_2 synthase.¹⁷ Stock solutions (300 mM) were prepared with DMSO and kept at -20°C until use. The applied concentrations of picotamide were based on previous studies performed with prostate and platelets.^{1,11,12,18} U46619

((Z)-7-[(1S,4R,5R,6S)-5-[(E,3S)-3-hydroxyoct-1-enyl]-3-oxabicyclo[2.2.1]heptan-6-yl]hept-5-enoic acid) is an agonist of the TXA_2 receptor,¹⁹ and was dissolved in ethanol. As thromboxane A_2 is highly unstable, U46619 is commonly used as a thromboxane A_2 receptor agonist.^{20,21} Stock solutions (10 mM) were stored at -80°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.¹⁹ Carbachol (carbamoylcholin) and methacholine are muscarinic acetylcholine receptor agonists.^{19,22} Adenosine-5'-triphosphate (ATP) is the endogenous ligand of purinergic P2X receptors, whereas α,β -methylene-adenosine 5'-triphosphate (α,β -methylene-ATP) is a full P2X1 and P2X3 agonist, but may also cause rapid desensitization and consequent inhibition of P2X1- and P2X3-mediated effects.²³ Aqueous stock solutions (10 mM) of noradrenaline, phenylephrine, methoxamine, carbachol, methacholine, and serotonin (5-hydroxytryptamine) were freshly prepared before each experiment. ATP (500 mM) were freshly prepared before each experiment and stored on ice until application to organ bath chambers. Stock solutions (10 mM) of α,β -methylene-ATP were prepared with water and stored at -20°C until used and before further dilution with water. Aqueous stock solutions of endothelin-1 (0.75 mM), angiotensin-II (1 mM), and [lys⁸]-vasopressin (500 μ M) were stored at -20°C as small aliquots, so that repeating freezing and thawing cycles were avoided. Picotamide, U46619, and endothelin-1 were obtained from Enzo Life Sciences (Lörrach, Germany). Noradrenaline, phenylephrine, methoxamine, carbachol, methacholine, serotonin, and ATP disodium salt were obtained from Sigma-Aldrich (Munich, Germany). α,β -Methylene-ATP and vasopressin were obtained from Tocris (Bristol, UK).

2.4 | Data and statistical analyses

Data in concentration and frequency response curves are presented as means \pm standard deviation (SD), with the indicated number (n) of independent experiments. Tissue from one animal was used for one independent experiment, so that the indicated numbers of experiments also reflect the number of animals, on which a given series is built up. For presentation of E_{\max} and EC_{50} values, single values from all experiments are shown in scatter plots. Maximum contractions are reported as means with 95% confidence intervals (CIs). Effect sizes become obvious from concentration and frequency response curves. Nevertheless, some exemplary values are additionally reported as mean differences (MD) with 95% CI, e.g. for inhibitions at maximum contractions or for maximum effects. One-way analysis of variance (ANOVA) was used for comparison of whole concentration/frequency response curves, and two-way ANOVA was used for comparison of contractions at single concentrations or frequencies. For comparison of E_{\max} and EC_{50} values, a paired Student's *t*-test was applied. Tests for concentration response curves and calculation of MD with 95% CI were performed using the SPSS[®] version 20 (IBM SPSS Statistics, IBM Corporation). Tests for paired comparison of E_{\max}

and EC_{50} values were performed using GraphPad Prism 6. $p < .05$ were considered significant. However, the present study and analyses were designed to be exploratory, but not designed to test a pre-specified statistical null hypothesis.²⁴ Apart from a lacking hypothesis, recently defined features imparting the character of hypothesis-testing study design were lacking in our study, including a clear preset study plan, blinding, or biometric calculation of groups sizes.²⁴ Therefore, p values reported here should be considered as descriptive and not as hypothesis-testing.²⁴ In line with recent recommendations, p values were used sparingly, and the focus in our study is on effect sizes.²⁴

According to recent guidelines for analyzing and reporting data, procedures and statistical methods, our procedures are described in detail as follows.²⁴ As a calculation of (descriptive) p values was intended, the minimum number of experiments and group sizes in organ bath experiments was pre-planned as $n = 5/\text{group}$ for each series. Data were analyzed, after at least five experiments of a series were performed. Following this analysis, series were discontinued if it became obvious that no effect could be expected on this data basis, or if descriptive $p < .05$ were obtained in concentration/frequency response curves (at single frequencies/agonist concentrations, and/or between whole groups). If these initial results appeared ambiguous, i.e. did not reveal $p < .05$, but suggested that an effect could be expected, series were continued and analyzed again. This procedure was possible due to the explorative character of this study, and as it is reported here in detail.²⁴ In fact, flexible group sizes have been recommended by guidelines for experimental design and analysis in experimental pharmacology, if data are characterized by large variations, what applies here.^{25,26} However, interim analyses were limited to concentration and frequency response curves and did not include E_{\max} and EC_{50} values, which were calculated by curve fitting following completion of series. According to the paired design (allocation of samples from each tissue to the control and inhibitor groups), groups being compared with each other by statistical tests showed identical group sizes. No data or experiments were excluded from analyses, apart from one value in a series addressing effects of DMSO and picotamide on phenylephrine-induced contractions of interlobar arteries. Thus, in the control group of one single experiment of this series, curve fitting revealed an EC_{50} value ranging >1500-fold higher than the highest of all other values in this group. Consequently, this value was regarded as an obvious outlier and excluded from diagrams and calculations, what is again mentioned in the legend to figures.

2.5 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <https://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY,²⁷ and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.^{19,23,28}

3 | RESULTS

3.1 | Maximum contractions

Effects of picotamide were examined on contractions induced by the thromboxane A_2 analog U46619, EFS, by three different α_1 -adrenoceptor agonists (noradrenaline, phenylephrine, methoxamine), two cholinergic agonists (methacholine, carbachol), serotonin, the purinergic agonists ATP and α,β -methylene-ATP, angiotensin-II, endothelin-1, and vasopressin. EFS induced frequency-dependent contractions in interlobar arteries (Figure 1A), but not in coronary arteries (data not shown). All three α_1 -adrenergic agonists induced concentration-dependent contractions in interlobar arteries, whereas only phenylephrine and methoxamine, but not noradrenaline induced contractions in coronary arteries (Figure 1A). Both cholinergic agonists induced concentration-dependent contractions in coronary arteries (Figure 1A), but were not applied to interlobar arteries. Both purinergic agonists induced contractions in interlobar, but not in coronary arteries (Figure 1A). Vasopressin induced no obvious contractions in both vessel types (Figure 1A). The remaining agonists induced contractions in both vessel types (Figure 1A), which were concentration-dependent for serotonin and angiotensin-II, but not for endothelin-1 in the applied concentration range.

In interlobar arteries, strongest contractions were observed for α_1 -adrenergic agonists and endothelin-1, all ranging roughly around threefold of EFS-induced contractions (Figure 1A). Serotonin- and U46619-induced contractions in interlobar arteries were in similar ranges as EFS-induced contractions (Figure 1A). Purinergic and angiotensin-II induced the lowest contractions, which were in similar ranges (Figure 1A). Vasopressin induced no contractions (Figure 1A). In coronary arteries, contractions were strongest following application of U46619 or endothelin-1 (Figure 1A). α_1 -Adrenergic contractions in coronary arteries were lower compared with interlobar arteries, whereas serotonin- and angiotensin-II-induced contractions were comparable between both vessel types (Figure 1A). Cholinergic contractions exceeded α_1 -adrenergic contractions in coronary arteries (Figure 1A). Maximum contractions in control groups (shown in Figure 1A) occurred at different frequencies and agonist concentrations (Table 1).

On average, contractions induced by 80 mM KCl amounted to 13.6 mN [12.0 to 15.1] in renal interlobar arteries, and to 28.8 mN [25.0 to 32.5] in coronary arteries. KCl-induced contractions were assessed before application of DMSO (in controls) or picotamide, and did not differ between groups allocated subsequently to control and picotamide groups (Figure 1B).

3.2 | U46619-induced contractions

U46619-induced contractions were inhibited by picotamide (300 μM) in interlobar and coronary arteries, without right shifts

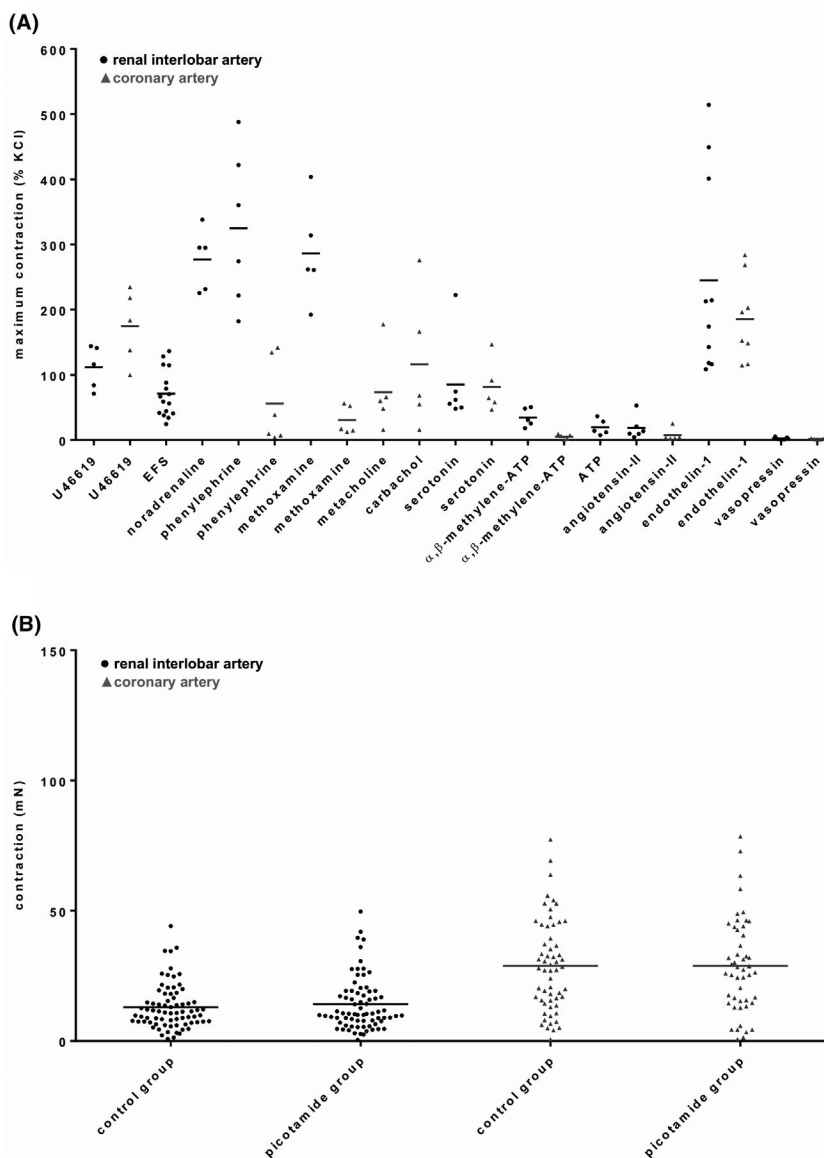


FIGURE 1 Maximum and highmolar KCl-induced contractions of porcine renal interlobar and coronary arteries. In (A), values are maximum contractions following application of EFS and agonists as indicated in control groups, regardless at which frequency or agonist concentration they occurred (for concentrations, see Table 1). In (B), contractions induced by highmolar KCl are expressed in mN. EFS- and agonist-induced contractions in all other diagrams have been calculated as percentage of contractions induced by 80 mM KCl. KCl-induced contractions were assessed before application of DMSO (in controls) or picotamide. Thus, contractions shown for controls and picotamide were in fact assessed under the same conditions, i.e. without DMSO or picotamide, but here shown separately to demonstrate that overall contractions were similar in picotamide and control groups. Shown are all single values from all experiments performed for frequency response curves and concentration response curves in Figures 2–5 together with means (=bars). Contractions were obtained under the presence of DMSO (controls, solvent for picotamide). As three concentrations of picotamide were examined for EFS-induced contractions, final concentrations of DMSO in organ bath chambers amounted to 0.1%, 0.33%, and 1% for EFS, and 1% in experiments with agonists.

and without clearly increased EC_{50} values ($p < .002$ for controls vs. picotamide in renal interlobar arteries; $p < 0.002$ in coronary arteries) (Figure 2A,B). Exemplarily, submaximum contractions induced by 100 nM U46619 were inhibited by 28% [9.7 to 25.5] in interlobar arteries, and by 24% [−16.7 to 63.9] in coronary arteries. E_{max} values for U46619 calculated by curve fitting of concentration response were reduced by trend by picotamide, whereas EC_{50} values of U46619 were not clearly changed (Figure 2A,B).

3.3 | EFS-induced contractions

Effects of picotamide on EFS-induced contractions of interlobar arteries were examined in three independent series, where three different concentrations of picotamide and corresponding amounts of solvent were applied. EFS-induced contractions were inhibited by 100 and 300 μ M of picotamide (Figure 2C,D). Inhibitions occurred at different frequencies, and were confirmed by comparisons of whole

TABLE 1 Distribution of maximum EFS- and agonist-induced contractions in porcine renal interlobar and coronary arteries across frequencies (EFS) and agonist-concentrations. Indicated are concentrations and frequencies inducing the maximum response in construction of concentration and frequency response curves, in at least one independent experiment, together with the number of experiments in which this concentration/frequency induced the maximum response and with the total number of experiments in each series.

	Renal interlobar	Coronary
U46619	Total: <i>n</i> = 5 1 μ M: <i>n</i> = 1 3 μ M: <i>n</i> = 5	Total: <i>n</i> = 5 100 nM: <i>n</i> = 1 300 nM: <i>n</i> = 2 10 μ M: <i>n</i> = 1 30 μ M: <i>n</i> = 1
EFS	Total: <i>n</i> = 16 16 Hz: <i>n</i> = 2 32 Hz: <i>n</i> = 3 64 Hz: <i>n</i> = 11	—
Noradrenaline	Total: <i>n</i> = 5 10 μ M: <i>n</i> = 5	—
Phenylephrine	Total: <i>n</i> = 6 3 μ M: <i>n</i> = 1 10 μ M: <i>n</i> = 1 30 μ M: <i>n</i> = 4	Total: <i>n</i> = 6 1 μ M: <i>n</i> = 2 10 μ M: <i>n</i> = 2 100 μ M: <i>n</i> = 2
Methoxamine	Total: <i>n</i> = 5 10 μ M: <i>n</i> = 3 100 μ M: <i>n</i> = 1 300 μ M: <i>n</i> = 1	Total: <i>n</i> = 5 100 μ M: <i>n</i> = 1 300 μ M: <i>n</i> = 1 1 mM: <i>n</i> = 3
Methacholine	—	Total: <i>n</i> = 5 1 μ M: <i>n</i> = 1 3 μ M: <i>n</i> = 3 10 μ M: <i>n</i> = 1
Carbachol	—	Total: <i>n</i> = 5 1 μ M: <i>n</i> = 1 10 μ M: <i>n</i> = 2 30 μ M: <i>n</i> = 1 100 μ M: <i>n</i> = 1
Serotonin	Total: <i>n</i> = 6 1 μ M: <i>n</i> = 3 3 μ M: <i>n</i> = 2 10 μ M: <i>n</i> = 10	Total: <i>n</i> = 5 3 μ M: <i>n</i> = 1 10 μ M: <i>n</i> = 1 30 μ M: <i>n</i> = 3
α , β -methylene-ATP	Total: <i>n</i> = 5 3 μ M: <i>n</i> = 1 10 μ M: <i>n</i> = 1 30 μ M: <i>n</i> = 3	Total: <i>n</i> = 5 100 nM: <i>n</i> = 1 10 μ M: <i>n</i> = 4
ATP	Total: <i>n</i> = 5 0.3 μ M: <i>n</i> = 1 3 μ M: <i>n</i> = 2 30 μ M: <i>n</i> = 2	—
Angiotensin-II	Total: <i>n</i> = 6 10 nM: <i>n</i> = 1 100 nM: <i>n</i> = 5	Total: <i>n</i> = 5 100 nM: <i>n</i> = 2 1 μ M: <i>n</i> = 3
Endothelin-1	Total: <i>n</i> = 10 10 nM: <i>n</i> = 1 30 nM: <i>n</i> = 6 300 nM: <i>n</i> = 3	Total: <i>n</i> = 8 30 nM: <i>n</i> = 1 100 nM: <i>n</i> = 3 300 nM: <i>n</i> = 1 1 μ M: <i>n</i> = 1 3 μ M: <i>n</i> = 2

frequency response curves to controls ($p < .02$ for 100 μ M picotamide, $p < .005$ for 300 μ M picotamide) (Figure 2C,D). Exemplarily, average maximum contractions in frequency response curves were inhibited by 32% [−4.6 to 68.3] at 64 Hz by 100 μ M picotamide, and by 48% [7.8 to 88.1] at 16 Hz by 300 μ M picotamide. E_{max} values calculated by curve fitting were reduced by trend, whereas frequencies inducing 50% of the maximum EFS-induced contraction were not affected (Figure 2C,D): 30 μ M picotamide did not change EFS-induced contractions (Figure 2E).

3.4 | α_1 -Adrenergic contractions

In interlobar arteries, picotamide (300 μ M) shifted concentration response curves for noradrenaline to the right, so that contractions were reduced at submaximal concentrations for noradrenaline, but recovered at high noradrenaline concentrations (Figure 2F). Accordingly, picotamide increased EC_{50} values for noradrenaline from 0.5 μ M [−0.006 to 0.952] in controls to 1.9 μ M [0.7 to 3.1] (MD 1.44 μ M [−0.366 to 2.518]), whereas E_{max} values calculated from curve fitting remained similar (Figure 2F).

In interlobar arteries, picotamide (300 μ M) shifted concentration response curves for phenylephrine and methoxamine to the right, so that contractions were reduced at submaximal agonist concentrations, but recovered at high agonist concentrations (Figure 3A,B). Accordingly, picotamide increased EC_{50} values for phenylephrine from 0.4 μ M [0.1 to 0.7] in controls to 3.1 μ M [1.2 to 5] (MD 2.7 [0.9 to 4.6]), and EC_{50} values for methoxamine from 1.1 μ M [−0.5 to 2.6] to 9.0 μ M [2.3 to 15.8] (MD 8.0 [2.2 to 13.7]) (Figure 3A,B). E_{max} values of both agonists were not affected by picotamide (Figure 3A,B).

In coronary arteries, picotamide inhibited phenylephrine- and methoxamine-induced contractions, without consistent changes of EC_{50} values and without recovery in the examined concentration range (phenylephrine: $p < .05$ for controls vs. picotamide; methoxamine: $p < .01$) (Figure 3C,D). Exemplarily, average maximum phenylephrine-induced contractions (at 1 mM phenylephrine) were inhibited by 58.3% [−25.6 to 97.6], and average maximum methoxamine-induced contractions (at 300 μ M methoxamine) by 46.1% [22.8 to 69.5] by picotamide (Figure 3C,D). In contrast to interlobar arteries, no rightshifts and no increases of EC_{50} values by picotamide were observed for phenylephrine or methoxamine in coronary arteries (Figure 3C,D). Noradrenaline did not induce contractions of coronary arteries (data not shown).

3.5 | Cholinergic contractions

In coronary arteries, picotamide (300 μ M) inhibited contractions induced by methacholine and carbachol, without consistent changes of EC_{50} values and without recovery in the examined concentration range (methacholine: $p < .001$ for controls vs. picotamide; carbachol: $p < .006$) (Figure 3E,F). Average maximum methacholine-induced

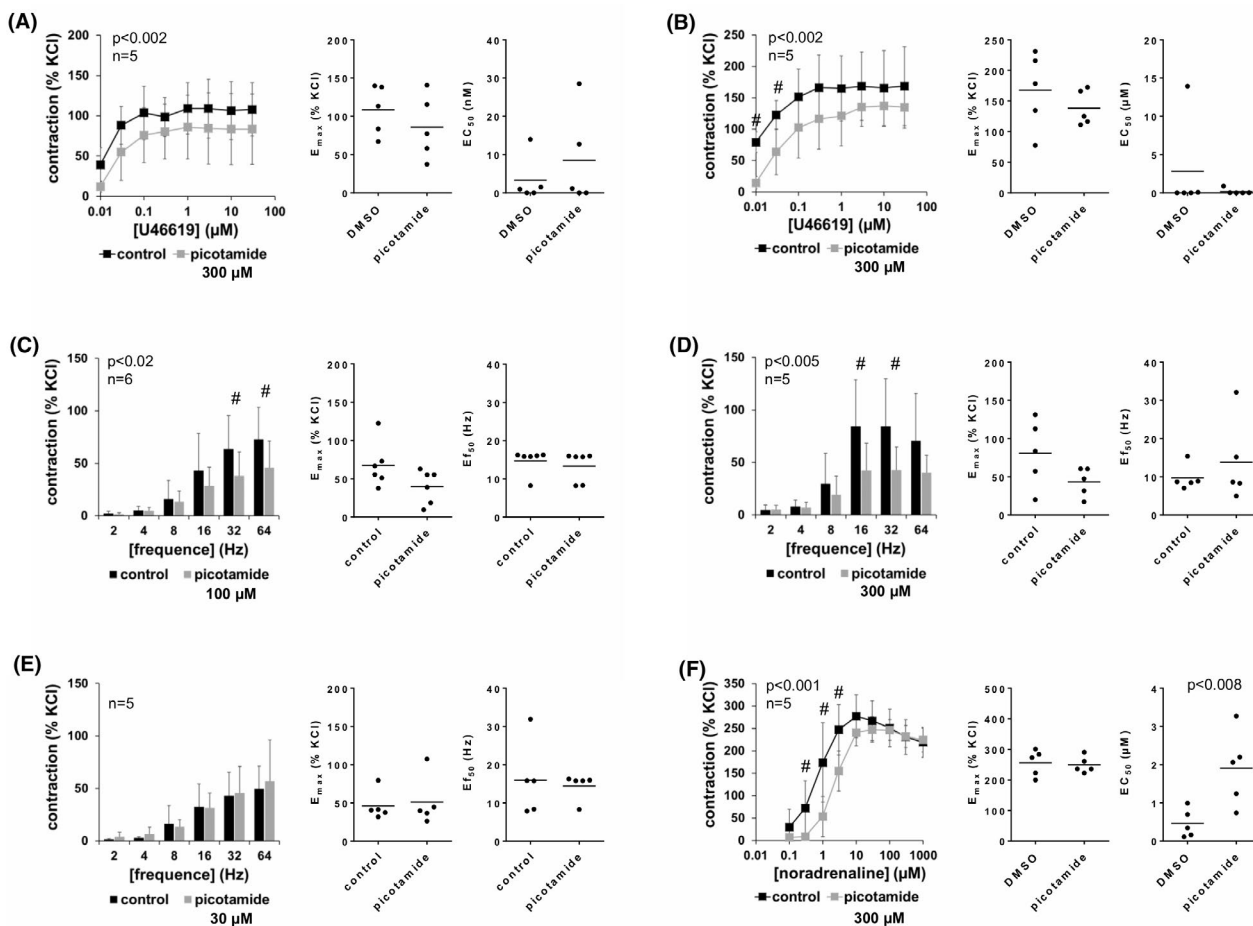


FIGURE 2 Effects of picotamide on U46619-, EFS-, and noradrenaline-induced contractions in renal interlobar (A, C-F) and coronary (B) arteries. Contractions were induced by cumulative concentrations of U46619, different frequencies of EFS, or by cumulative concentrations of noradrenaline in interlobar arteries, 30 min following administration of picotamide or solvent (controls). Shown are means \pm SD in frequency response curves ($\#p < .05$ for control vs. picotamide by two-way ANOVA, and p values for whole groups from one-way ANOVA in inserts), and all single E_{max} values and frequencies inducing 50% of the maximum EFS-induced contraction (E_{f50}) or EC_{50} values for noradrenaline from all experiments (calculated by curve fitting) in scatter plots (p value from paired Student's t -test), from experiments using tissues from $n = 5$ animals in A, B and D-F, and $n = 6$ animals in C. For each single experiment, tissue from one animal was allocated to the control and the picotamide group, which were examined in the same experiment.

contractions (at 3 μ M methacholine) were inhibited by 82% [76.9 to 87.6], and average maximum carbachol-induced contractions (at 10 μ M carbachol) by 70% [46.7 to 92.6] by picotamide (Figure 3E,F). E_{max} values calculated from frequency response curves were reduced by trend (Figure 3E,F).

3.6 | Serotonin-induced contractions

Serotonin-induced contractions were inhibited by picotamide (300 μ M) in interlobar and coronary arteries ($p < .03$ for controls vs. picotamide in renal interlobar arteries; $p < .001$ in coronary arteries) (Figure 4A,B). Average maximum contractions occurred at 3 μ M serotonin in interlobar arteries, which were inhibited by 72% [41.8 to 103] in interlobar arteries, and at 30 μ M in coronary arteries, which were inhibited by 52% [-3.2 to 107.7]. E_{max} values for serotonin calculated by curve fitting of concentration response were reduced by trend by picotamide (Figure 4A,B). EC_{50} values for serotonin were

increased by picotamide from 0.248 μ M [-0.196 to 0.692] in controls to 10.9 μ M [-9.6 to 31.3] (MD 10.6 [-7.2 to 28.4]) in interlobar arteries, and from 0.605 μ M [-0.422 to 1.633] to 9.584 μ M [3.285 to 15.88] (MD 8.98 μ M [3.677 to 14.28]) in coronary arteries (Figure 4A,B).

3.7 | Purinergic contractions

α,β -Methylene-induced contractions were inhibited by picotamide (300 μ M) in interlobar arteries ($p < .03$ for controls vs. picotamide), at the two highest applied concentrations of α,β -methylene-ATP (Figure 4C). Thus, contractions induced by 10 μ M α,β -methylene-ATP were inhibited by 65% [13 to 116], whereas contractions induced by 30 μ M were inhibited by 22% [-94 to 138]. ATP-induced contractions were weak and hardly lower in the presence of picotamide, although statistical testing suggested an inhibition ($p < .04$) (Figure 4C). Calculation of conclusive E_{max} and EC_{50} values was not

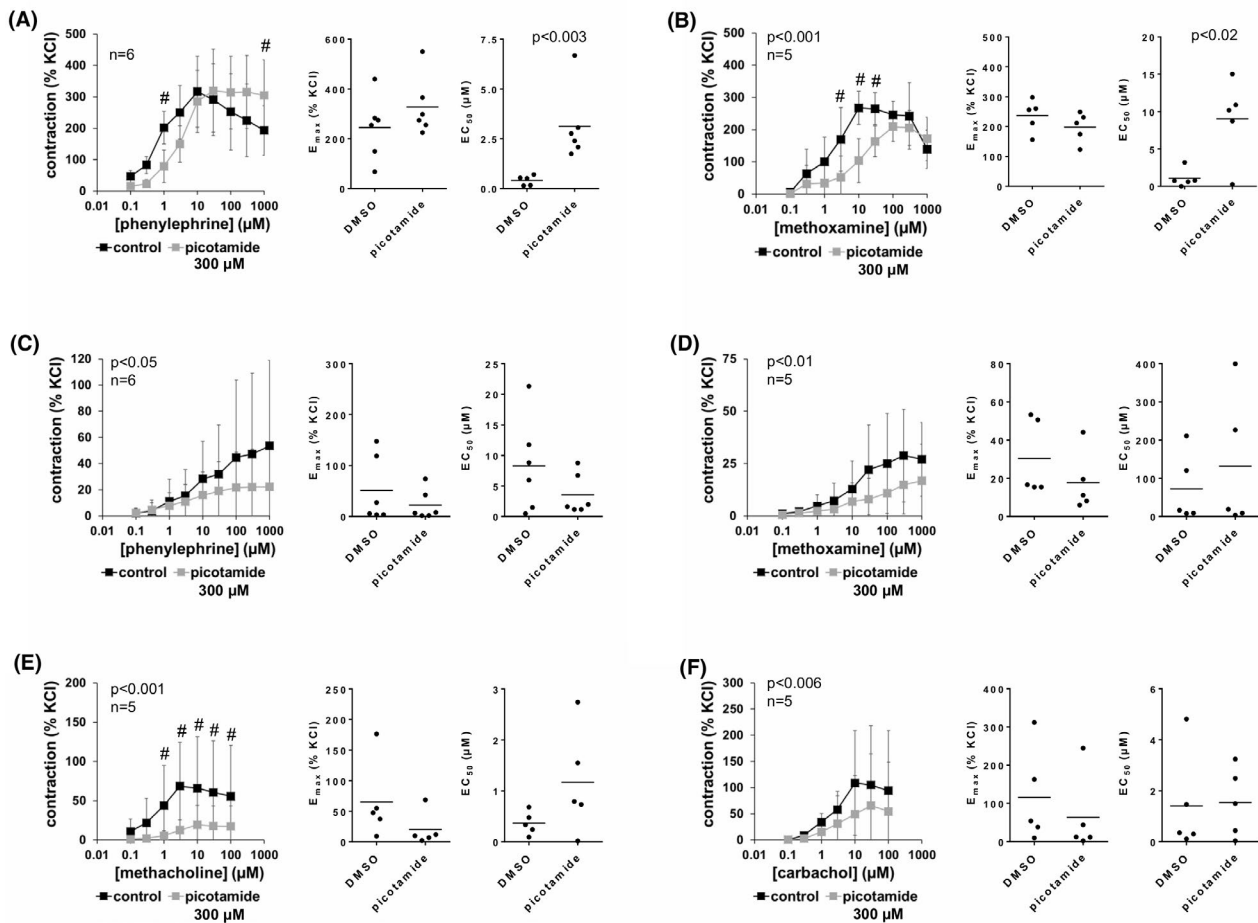


FIGURE 3 Effects of picotamide on α_1 -adrenergic and cholinergic contractions in renal interlobar and coronary arteries. Contractions were induced by cumulative concentrations of α_1 -adrenergic agonists (phenylephrine, methoxamine) or cholinergic agonists (methacholine, carbachol) in interlobar arteries (A, B) or coronary arteries (C-F), 30 min following administration of picotamide (300 μM) or solvent (controls). Shown are means \pm SD in concentration response curves (# $p < .05$ for control vs. picotamide by two-way ANOVA, and p values for whole groups from one-way ANOVA in inserts), and all single E_{max} and EC_{50} values for single experiments (calculated by curve fitting) in scatter plots (p value from paired Student's t -test), from experiments using tissues from $n = 5$ animals in B, D, E and F, and $n=6$ animals in A and C. For each single experiment, tissue from one animal was allocated to the control and the picotamide group, which were examined in the same experiment. In one of six experiments performed for the series in (A), curve fitting revealed an EC_{50} value of 756 μM for phenylephrine in the control group, which has been omitted from the diagram and calculations. For each single experiment, tissue from one animal was allocated to the control and the picotamide group, which were examined in the same experiment.

possible, as curve fitting was not possible for several single experiments using α,β -methylene-ATP, and a sigmoidal character was not given, and as clear contraction-concentration relationships were lacking for ATP (Figure 4D). No contractions were observed in response to both agonists in coronary arteries (Figure 4D). ATP even induced relaxations of basal tone in coronary arteries, which was not changed by picotamide (Figure 4D).

3.8 | Angiotensin-II-induced contractions

Angiotensin-II-induced contractions were inhibited by picotamide (300 μM) in interlobar and coronary arteries ($p < .008$ for controls vs. picotamide in renal interlobar arteries; $p < .04$ in coronary arteries) (Figure 5A,B). Average maximum contractions occurred at 100 nM angiotensin-II in interlobar arteries, which were inhibited

by 42% [0.4 to 82.9] in interlobar arteries, and at 1 μM in coronary arteries, which were inhibited by 60% [38.3 to 80.8]. E_{max} values for angiotensin-II calculated by curve fitting of concentration response were reduced by trend by picotamide (Figure 5A,B). EC_{50} values for angiotensin-II were not clearly affected by picotamide (Figure 5A,B).

3.9 | Endothelin-1-induced contractions

Endothelin-1-induced contractions were inhibited by picotamide (300 μM) in interlobar and coronary arteries ($p < .008$ for controls vs. picotamide in renal interlobar arteries; $p < .002$ in coronary arteries) (Figure 5C,D). Due to lacking concentration-dependent relationships of endothelin-1-induced contractions, curve fitting was not possible (Figure 5C,D). Exemplarily, contractions induced by 1 μM

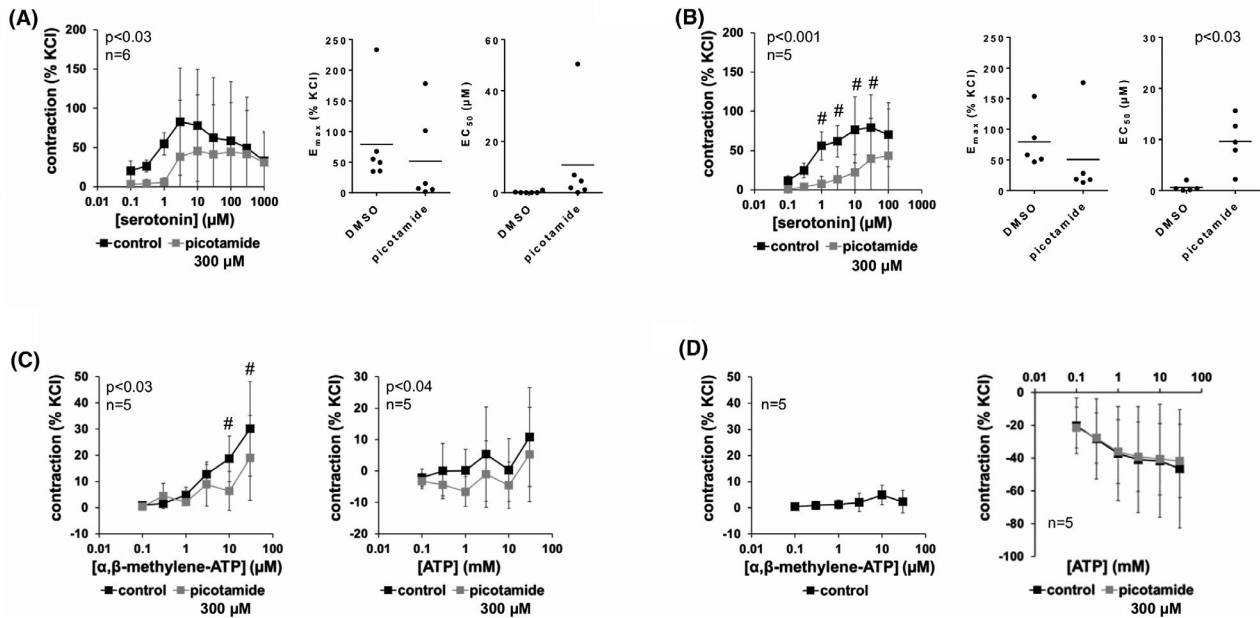


FIGURE 4 Effects of picotamide on serotonin-induced and purinergic contractions in renal interlobar and coronary arteries. Contractions were induced by cumulative concentrations of serotonin (A, B) and α,β -methylene-ATP and ATP (C, D) in interlobar arteries (A, C) or coronary arteries (B, D), 30 min following administration of picotamide (300 μM) or solvent (controls) (with the exception of (D), where not picotamide was included in experiments with α,β -methylene-ATP). Shown are means \pm SD in concentration response curves ($\#p < .05$ for control vs. picotamide by two-way ANOVA, and p values for whole groups from one-way ANOVA in inserts), and (p value from paired Student's t -test), from experiments using tissues from $n = 5$ animals in B-D, and $n = 6$ animals in A.

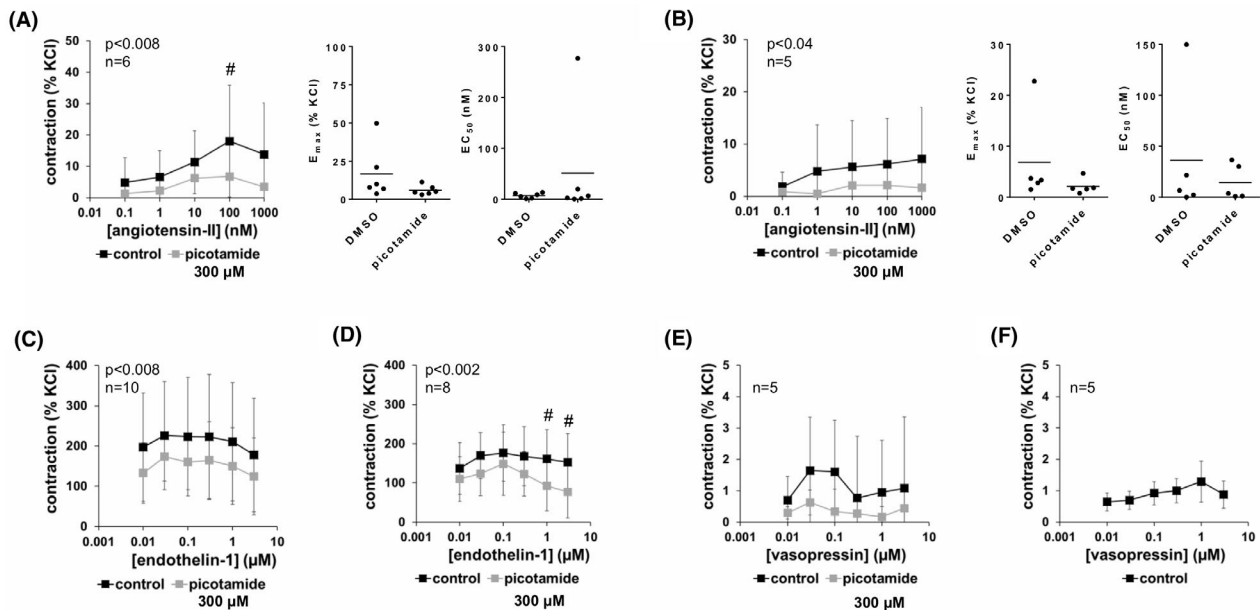


FIGURE 5 Effects of picotamide on angiotensin-II-, endothelin-1-, and vasopressin-induced contraction in renal interlobar and coronary arteries. Contractions were induced by cumulative concentrations of angiotensin-II (A, B), endothelin-1 (C, D), or vasopressin (E, F) in interlobar arteries (A, C, E) or coronary arteries (B, D, F), 30 min following administration of picotamide (300 μM) or solvent (controls), with the exception of (E). Shown are means \pm SD in concentration response curves ($\#p < .05$ for control vs. picotamide by two-way ANOVA, and p values for whole groups from one-way ANOVA in inserts), and all single E_{max} and EC_{50} values for single experiments (calculated by curve fitting) in scatter plots (p value from paired Student's t -test), from experiments using tissues from $n = 5$ animals in B, E, and F, $n = 6$ animals in A, $n = 8$ animals in D, and $n = 10$ animals in C. For each single experiment, tissue from one animal was allocated to the control and the picotamide group, which were examined in the same experiment.

endothelin-1 were inhibited by 24% [10.6 to 37.9] by picotamide in interlobar arteries, and by 44% [25.3 to 62.2] in coronary arteries.

3.10 | Vasopressin-induced contractions

Vasopressin did not induce contractions in interlobar and coronary arteries (Figure 5E-F). Consequently, results obtained under the presence of picotamide in interlobar arteries are inconclusive (Figure 5E), and no curves with picotamide were recorded in coronary arteries (Figure 5F).

4 | DISCUSSION

Previous studies addressing inhibition of smooth muscle contraction suggested effects of the combined thromboxane A_2 receptor antagonist and thromboxane A_2 synthase inhibitor picotamide on smooth muscle contractions, induced by agonists other than thromboxane A_2 . Besides thromboxane A_2 -induced contractions, picotamide inhibited α_1 -adrenergic, cholinergic, and serotonin-induced contractions of rodent aortic tissues.^{13,14} Data for other vasoconstrictors, other vessel types, or other species were to the best of knowledge not available. Here, we examined effects of picotamide on a broad panel of vasoconstrictors and on neurogenic contractions in renal interlobar arteries, and in coronary arteries. We observed that picotamide inhibited contractions induced by a wide spectrum of

vasoconstrictors and by neurogenic stimulation in both vessel types. The pattern of inhibition differed between vasoconstrictors and vessel types (Table 2).

Regarding the inhibition of U46619-induced contractions, the lacking right shift may be surprising. As picotamide has been originally described as a thromboxane A_2 receptor antagonist, a corresponding inhibition pattern should be expected, including right shifts of concentration response curves and increases of EC_{50} values for U46619, as described for rabbit aortic rings.¹³ On the other hand, inhibition of U46619-induced contractions without patterns of competitive agonists has been reported from human trigone tissues, and partially from human prostate tissues.^{11,12} It has been suggested, that features of competitive antagonism may be covered by non-competitive antagonism, if both occur together. In fact, non-competitive inhibition of vasoconstrictor effects, and irreversible and insurmountable antagonism of thromboxane A_2 receptors by picotamide have been previously proposed, in addition to competitive antagonism.^{18,29} Such indisplaceable binding may result from stable interactions between picotamide and thromboxane A_2 receptors, what was observed in platelets following incubation periods exceeding 20 min.^{18,29} Irreversible, insurmountable antagonism, occurring alone or together with competitive antagonism, may be characterized by reduced E_{max} values, and was supposed to account for effects of picotamide on receptors other than thromboxane A_2 receptors.^{12,30,31} Insurmountable antagonism may have occurred in our experiments, as we applied picotamide for 30 min. However, certain identification of true competitive and non-competitive antagonism

TABLE 2 Effects of picotamide on agonist- and EFS-induced contractions of porcine renal interlobar and coronary arteries, summary and simplification of present finding

	Renal interlobar	Coronary
U46619	Reduced contraction in concentration response curves. EC_{50} and E_{max} unchanged	Reduced contraction in concentration response curves. EC_{50} and E_{max} unchanged
EFS	Inhibited by 100 and 300 μ M picotamide, but not 30 μ M	—
Noradrenaline	Right shift of concentration response curve. EC_{50} increased, E_{max} unchanged	—
Phenylephrine	Right shift of concentration response curve. EC_{50} increased, E_{max} unchanged	Reduced contraction in concentration response curves. EC_{50} not increased
Methoxamine	Right shift of concentration response curve. EC_{50} increased, E_{max} unchanged	Reduced contraction in concentration response curves. EC_{50} not clearly increased
Methacholine	—	Reduced contraction in concentration response curves. EC_{50} increased, E_{max} reduced
Carbachol	—	Reduced contraction in concentration response curves. E_{max} slightly reduced
Serotonin	Reduced contraction in concentration response curves, possible right shift. EC_{50} increased	Reduced contraction in concentration response curves, possible right shift. EC_{50} increased
α,β -methylene-ATP	Reduced contractions at high agonist concentrations. No curve fitting	Contractions very low, <10% of KCl in control group
ATP	Contractions very low, <10% of KCl in control group	—
Angiotensin-II	Reduced contractions at high agonist concentrations. EC_{50} not clearly changed	Reduced contractions at high agonist concentrations. EC_{50} not clearly changed
Endothelin-1	Reduced contraction in concentration response curves. No curve fitting	Reduced contraction in concentration response curves. No curve fitting

requires extended experimental settings and is not possible on the basis of our data.^{30–32}

In interlobar arteries, picotamide shifted concentration response curves for contractions induced by α_1 -adrenergic agonists to the right, resulting in increased EC_{50} values for agonists without changes in maximum contractions. This pattern was confirmed using three different α_1 -adrenergic agonists. Although these are features of competitive antagonism, our data are not fully conclusive to this regard. Binding studies were not performed here, but would be required to confirm competitive antagonism of α_1 -adrenoceptors by picotamide. In coronary arteries, phenylephrine- and methoxamine-induced contractions were inhibited by picotamide as well, but without features of competitive antagonism. Partially, this is in line with previous findings suggesting inhibition of α_1 -adrenergic contractions by picotamide without right shift in rabbit aortic rings.¹³ In addition to vasoconstriction, inhibition of α_1 -adrenergic smooth muscle contraction by picotamide has been reported from human prostate and trigone tissues.¹² However, these studies did not include agonist concentrations higher than 100 μM , which may be required to observe recovery and curve shifts in competitive antagonism.

Apart from thromboxane A_2 and α_1 -adrenoceptors, vascular smooth muscle contraction can be induced by serotonin, angiotensin-II, and endothelin-1. All of these contractions were inhibited by picotamide, although to different extent and by divergent patterns (Table 2). Our data for serotonin are characterized by high variations of contraction levels between experiments using interlobar arteries, and are limited by the applied concentration range in coronary arteries. With all due caution, a right shift may be assumed considering our concentration response curves for serotonin. Regarding speculations about a competitive antagonism, the same limitations as for α_1 -adrenoceptor-agonists explained above apply here again. For endothelin-1, a clear relationship between concentration and contraction was lacking in our applied concentration range. In line with our current findings, inhibition of endothelin-induced contractions has been reported from human trigone tissues.¹²

Although vasorelaxation is the predominant outcome of cholinergic regulation in blood vessels, cholinergic vasoconstriction occurs in vessels with defective or lacking endothelium and has been repeatedly reported from coronary arteries. We induced cholinergic contractions of coronary arteries using methacholine and carbachol, which were both inhibited by picotamide. In isolated aortic rings from spontaneously hypertensive rats, where the release of nitric oxide was inhibited using a nitric oxide inhibitor, acetylcholine-induced contractions were inhibited by picotamide.¹⁴

Picotamide inhibited EFS-induced contractions using concentrations of 100 and 300 μM , but not 30 μM . Previous studies reported varying K_D , IC_{50} , and EC_{50} values of picotamide, which were obtained using different models. It has been proposed, that picotamide binding to receptors stabilizes over time, resulting in turnover from displaceable to non-displaceable binding, and in a high range of K_D , IC_{50} , and EC_{50} values.¹⁸ In radioligand binding assays in human platelets, thromboxane A_2 receptor agonists were replaced by picotamide with K_D values of 0.38 or 1.5 μM .^{1,18} In vitro, U46619-induced

platelet aggregation was inhibited with time-dependent IC_{50} values, ranging from 4.7 to 450 μM .¹⁸ Other studies addressing effects of picotamide on platelet aggregation used concentrations of 100–500 μM and reported IC_{50} values in these ranges.^{13,17,29,33,34} In cultured human and rat aortic smooth muscle cells, picotamide inhibited migration and growth factor-induced proliferation in one to low two-digit ranges.³⁵ In the same study, U46619-induced proliferation was not inhibited by picotamide concentrations up to 50 μM , but by 500 μM .³⁵ In addition to thromboxane A_2 receptor antagonism, picotamide acts as an inhibitor of thromboxane A_2 synthase,² and consequently reduces plasma thromboxane A_2 levels in patients.³⁶ In vitro, picotamide inhibits thromboxane A_2 formation by platelets, with IC_{50} values ranging from 0.43 to 54 μM ¹⁸ or up to 140 μM .¹⁷ According to these previous reports, we applied picotamide in a concentration of 300 μM in our previous studies, which inhibited contractions of human prostate and trigone tissues.^{11,12} In our present study, 100 and 300 μM appeared equally effective, whereas 30 μM were without effect on EFS-induced contractions. Together, our findings are in line with previous studies suggesting that functional picotamide effects in vitro require concentrations in a three-digit micromolar range. Our current and previous observations contrast K_D values for picotamide binding to thromboxane A_2 receptors and clinical observations. Thus, oral administration of a standard dose of picotamide (300 mg) results in peak plasma levels of around 5.4 μM .³⁷ However, antiplatelet activity and inhibition of TXA_2 administration by picotamide in patients do not correlate with plasma concentrations of picotamide.³⁷ Administration is safe and possible in daily cumulative doses up to 1500 mg per day.^{33,38}

Inhibition of thromboxane A_2 synthase may be involved as well in our findings, and may account for inhibition of different vasoconstrictors without off-target effects. Biosynthesis of thromboxane A_2 involves formation of arachidonic acid by phospholipase A_2 (PLA_2), which is processed to prostaglandins and finally to thromboxane A_2 by thromboxane A_2 synthase.² Activation of PLA_2 isoforms by contractile agonists and agonist-induced formation of arachidonic acid or thromboxane A_2 have been reported from different smooth muscle types. α_1 -Adrenoceptors, angiotensin-II, and serotonin activate PLA_2 and cause arachidonic acid formation in vascular smooth muscle cells, what may contribute to receptor-mediated vasoconstriction.^{39–43} PLA_2 activation by muscarinic receptors, followed by arachidonic acid formation and subsequent smooth muscle contraction by thromboxane A_2 has been suggested for airway and esophageal smooth muscle.^{44,45} Endothelin-1 may activate PLA_2 in a variety of cell types, including iris sphincter smooth muscle cells and glomerular smooth muscle cells,^{46,47} but is obviously not involved in vasoconstriction.^{48,49} Consequently, inhibition of contractions in our study must not necessarily reflect off-target effects of picotamide, but may (at least partially) result from inhibition of vasoconstrictor-induced thromboxane A_2 synthesis. In line with this, the dual thromboxane A_2 receptor antagonist and thromboxane A_2 synthase inhibitor picotamide previously inhibited EFS-induced contractions of prostate smooth muscle, what was not observed using pure thromboxane A_2 antagonists.¹¹

Assuming that agonist-induced release of thromboxane A_2 is involved in contractions of some vasoconstrictors, it follows that portions exceeding U46619-induced contractions can not be mediated by thromboxane A_2 release. In interlobar arteries, α_1 -adrenergic and endothelin-1-induced contractions clearly exceeded U46619-induced contractions. Consequently, the exceeding portion is most likely not mediated by thromboxane A_2 -release and occurs thromboxane A_2 -independently. Whether the other, non-exceeding components are in fact mediated by thromboxane A_2 release, cannot be confirmed on the basis of our data. None of the other agonists, and no agonist in coronary arteries induced stronger contractions than U46619. Some contractions were weaker than U46619-induced contractions, so that the amount of any released thromboxane A_2 (if involved at all) is not sufficient to induce a full thromboxane A_2 -induced contraction.

Our findings obtained from interlobar arteries are in line with clinical observations. In patients with congestive heart failure, picotamide improved renal perfusion and renal function, which was explained by decreased renal vascular resistance.³ Chronic congestive heart failure includes enhanced release of several vasoconstrictors, including endothelins and angiotensin,³ which were inhibited by picotamide in interlobar and coronary arteries in our study. In other studies, picotamide improved intrarenal blood flow and renal function in patients with type II diabetes.⁵⁰⁻⁵² This has been referred to improved renal blood flow resulting from antiaggregatory effects, as no data on renal vascular resistance were available.⁵⁰⁻⁵² Our current study suggests that previously observed improvements of kidney function occurred by inhibition of intrarenal vasoconstriction. Regarding that picotamide inhibits vasoconstriction in at least three vessel types, effects of picotamide on other vessel types, e.g. resistance vessels being critical for arterial blood pressure regulation appear possible as well. However, picotamide does not affect systolic or diastolic blood pressure.^{3,7,52} Evidence suggesting decreases in mean arterial blood pressure is to the best of our knowledge limited to mice.⁵³ Finally, inhibition of vasoconstriction may contribute to reduced numbers of vascular deaths, which was observed in clinical trials using picotamide and was previously explained by antiaggregatory effects.⁷

5 | CONCLUSIONS

Together, our findings suggest that picotamide inhibits contractions induced by a broad panel of vasoconstrictors in porcine interlobar and coronary arteries. The pattern of inhibition differed between vasoconstrictors and vessel types. Our findings from interlobar arteries are in line with previous observations, suggesting decreases of renal vascular resistance and improvements of renal blood flow and renal function in clinical studies.

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DISCLOSURE

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

Bingsheng Li performed methodology and model creation, study design, data analyses, investigation, writing of the original draft and visualization, project administration, and funding acquisition. **Ru Huang** performed methodology and model creation, study design, data analyses, investigation, and critical revision of the manuscript. **Ruixiao Wang** performed investigation and critical revision of the manuscript. **Yuhan Liu** performed investigation and critical revision of the manuscript. **Christian G. Stief** performed conceptualization, resources, and funding acquisition. **Martin Hennenberg** performed conceptualization, methodology and model creation, study design, data analyses, investigation, writing of the original draft and visualization, supervision and project administration, and funding acquisition.

ETHICS STATEMENT

This study was performed using arteries obtained from domestic animals, bred and sacrificed for meat production, i.e. from pig kidneys and pig hearts obtained from a local slaughterhouse by a butcher. Consequently, no ethical approval and no approval for animal experiments was required.

DATA AVAILABILITY STATEMENT

The raw data of this study are available from the corresponding author upon reasonable request. All data that support the findings of this study are included in the article.

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REFERENCES

1. Modesti PA, Colella A, Abbate R, et al. Competitive inhibition of platelet thromboxane A_2 receptor binding by picotamide. *Eur J Pharmacol.* 1989;169(1):85-93.
2. Nakahata N. Thromboxane A_2 : physiology/pathophysiology, cellular signal transduction and pharmacology. *Pharmacol Ther.* 2008;118(1):18-35.
3. Castellani S, Panizza R, Di Serio C, et al. Thromboxane inhibition improves renal perfusion and excretory function in severe congestive heart failure. *J Am Coll Cardiol.* 2003;42(1):133-139.
4. Celestini A, Violi F. A review of picotamide in the reduction of cardiovascular events in diabetic patients. *Vasc Health Risk Manag.* 2007;3(1):93-98.
5. Coccozza M, Milani M, Picano T, et al. Antiaggregatory effects of picotamide in long-term treatment: a 2-year, double-blind placebo-controlled trial. *Vasc Med.* 1997;2(4):292-295.
6. Hackam DG, Eikelboom JW. Antithrombotic treatment for peripheral arterial disease. *Heart.* 2007;93(3):303-308.

7. Nerisneri G, Coccheri S, Marubini E, et al. Picotamide, a combined inhibitor of thromboxane A2 synthase and receptor, reduces 2-year mortality in diabetics with peripheral arterial disease: the DAVID study. *Eur Heart J*. 2004;25(20):1845-1852.
8. Capra V, Bäck M, Angiolillo DJ, et al. Impact of vascular thromboxane prostanoid receptor activation on hemostasis, thrombosis, oxidative stress, and inflammation. *J Thromb Haemost*. 2014;12(2):126-137.
9. De Carlo M, Di Minno G, Sayre T, et al. Efficacy and safety of antiplatelet therapies in symptomatic peripheral artery disease: a systematic review and network meta-analysis. *Curr Vasc Pharmacol*. 2020.
10. Sharma AN, Deyell JS, Sharma SN, et al. Role of and recent evidence for antiplatelet therapy in prevention of cardiovascular disease in diabetes. *Curr Cardiol Rep*. 2019;21(8):78.
11. Hennenberg M, Miljak M, Herrmann D, et al. The receptor antagonist picotamide inhibits adrenergic and thromboxane-induced contraction of hyperplastic human prostate smooth muscle. *Am J Physiol Renal Physiol*. 2013;305(10):F1383-F1390.
12. Hennenberg M, Tamalunas A, Wang Y, et al. Inhibition of agonist-induced smooth muscle contraction by picotamide in the male human lower urinary tract outflow region. *Eur J Pharmacol*. 2017;803:39-47.
13. Vezza R, Spina D, Tallarida RJ, et al. Antivasoconstrictor and antiaggregatory activities of picotamide unrelated to thromboxane A2 antagonism. *Thromb Haemost*. 1997;78(5):1385-1391.
14. Abeywardena MY, Head RJ. Dietary polyunsaturated fatty acid and antioxidant modulation of vascular dysfunction in the spontaneously hypertensive rat. *Prostaglandins Leukot Essent Fatty Acids*. 2001;65(2):91-97.
15. Oelke M, Bachmann A, Descazeaud A, et al. EAU guidelines on the treatment and follow-up of non-neurogenic male lower urinary tract symptoms including benign prostatic obstruction. *Eur Urol*. 2013;64(1):118-140.
16. Erdogan BR, et al. Normalization of organ bath contraction data for tissue specimen size: does one approach fit all? *Naunyn Schmiedebergs Arch Pharmacol*. 2020;393(2):243-251.
17. Gresle P, Deckmyn H, Arnout J, et al. Characterization of N, N'-bis(3-picolyl)-4-methoxy-isophthalamide (picotamide) as a dual thromboxane synthase inhibitor/thromboxane A2 receptor antagonist in human platelets. *Thromb Haemost*. 1989;61(3):479-484.
18. Modesti PA, Cecioni I, Colella A, et al. Binding kinetics and antiplatelet activities of picotamide, a thromboxane A2 receptor antagonist. *Br J Pharmacol*. 1994;112(1):81-86.
19. Alexander SPH, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: G protein-coupled receptors. *Br J Pharmacol*. 2019;176(Suppl 1):S21-S141.
20. Malmsten C. Some biological effects of prostaglandin endoperoxide analogs. *Life Sci*. 1976;18(2):169-176.
21. Shen RF, Tai HH. Thromboxanes: synthase and receptors. *J Biomed Sci*. 1998;5(3):153-172.
22. Pei XF, et al. δ -beta-Acetylnortropine: a potent muscarinic agonist with apparent selectivity toward M2-receptors. *J Med Chem*. 1998;41(12):2047-2055.
23. Alexander SPH, Mathie A, Peters JA, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: ion channels. *Br J Pharmacol*. 2019;176(Suppl 1):S142-S228.
24. Michel MC, Murphy TJ, Motulsky HJ. New author guidelines for displaying data and reporting data analysis and statistical methods in experimental biology. *Mol Pharmacol*. 2020;97(1):49-60.
25. Curtis MJ, Alexander S, Cirino G, et al. Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers. *Br J Pharmacol*. 2018;175(7):987-993.
26. Curtis MJ, Bond RA, Spina D, et al. Experimental design and analysis and their reporting: new guidance for publication in BJP. *Br J Pharmacol*. 2015;172(14):3461-3471.
27. Harding SD, Sharman JL, Faccenda E, et al. The IUPHAR/BPS Guide to PHARMACOLOGY in 2018: updates and expansion to encompass the new guide to IMMUNOPHARMACOLOGY. *Nucleic Acids Res*. 2018;46(D1):D1091-D1106.
28. Alexander SPH, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: enzymes. *Br J Pharmacol*. 2019;176(Suppl 1):S297-S396.
29. Pulcinelli FM, Pignatelli P, Pesciotti M, et al. Mechanism of the persisting TxA2 receptor antagonism by picotamide. *Thromb Res*. 1997;85(3):207-215.
30. Vauquelin G, Van Liefde I, Birzbier BB, et al. New insights in insurmountable antagonism. *Fundam Clin Pharmacol*. 2002;16(4):263-272.
31. Vauquelin G, Van Liefde I, Vanderheyden P. Models and methods for studying insurmountable antagonism. *Trends Pharmacol Sci*. 2002;23(11):514-518.
32. Kenakin T. Overview of receptor interactions of agonists and antagonists. *Curr Prot Pharmacol*. 2008;42:4.
33. Berrettini M, De Cunto M, Parise P, et al. "In vitro" and "ex vivo" effects of picotamide, a combined thromboxane A2-synthase inhibitor and -receptor antagonist, on human platelets. *Eur J Clin Pharmacol*. 1990;39(5):495-500.
34. Cattaneo M, Tenconi PM, Lecchi A, et al. In vitro effects of picotamide on human platelet aggregation, the release reaction and thromboxane B2 production. *Thromb Res*. 1991;62(6):717-724.
35. Ratti S, Quarato P, Casagrande C, et al. Picotamide, an antithromboxane agent, inhibits the migration and proliferation of arterial myocytes. *Eur J Pharmacol*. 1998;355(1):77-83.
36. Danese C, Sergio G, Giunta G, et al. Effects of prolonged picotamide therapy on platelet activity in patients with peripheral arterial disease. *Curr Med Res Opin*. 1988;11(4):221-226.
37. Modesti PA. Picotamide: an inhibitor of the formation and effects of TXA₂. *Cardiovasc Drug Rev*. 1995;13(4):353-364.
38. Aliberti G, Proietta M, Pulignano I. Platelet and hemocoagulative changes in elderly atherosclerotic patients after treatment with the antiaggregating drug picotamide. *Arch Gerontol Geriatr*. 1997;25(2):193-200.
39. Gailly P, Gong MC, Somlyo AV, et al. Possible role of atypical protein kinase C activated by arachidonic acid in Ca²⁺ sensitization of rabbit smooth muscle. *J Physiol*. 1997;500(Pt 1):95-109.
40. Lawandy I, Liu Y, Shi G, et al. Increased coronary arteriolar contraction to serotonin in juvenile pigs with metabolic syndrome. *Mol Cell Biochem*. 2019;461(1-2):57-64.
41. Muthalif MM, Benter IF, Uddin MR, Malik KU. Calcium/calmodulin-dependent protein kinase II α mediates activation of mitogen-activated protein kinase and cytosolic phospholipase A2 in norepinephrine-induced arachidonic acid release in rabbit aortic smooth muscle cells. *J Biol Chem*. 1996;271(47):30149-30157.
42. Muthalif MM, Karzoun NA, Benter IF, et al. Functional significance of activation of calcium/calmodulin-dependent protein kinase II in angiotensin II-induced vascular hyperplasia and hypertension. *Hypertension*. 2002;39(2 Pt 2):704-709.
43. Rao GN, Lassègue B, Alexander RW, et al. Angiotensin II stimulates phosphorylation of high-molecular-mass cytosolic phospholipase A2 in vascular smooth-muscle cells. *Biochem J*. 1994;299(Pt 1):197-201.
44. Kim N, Sohn UD, Mangannan V, et al. Leukotrienes in acetylcholine-induced contraction of esophageal circular smooth muscle in experimental esophagitis. *Gastroenterology*. 1997;112(5):1548-1558.
45. Takata Y, Nishimura Y, Maeda H, et al. Phospholipase A2 augments contraction and intracellular calcium mobilization through thromboxane A2 in bovine tracheal smooth muscle. *Eur Respir J*. 1999;14(2):396-404.
46. Husain S, Abdel-Latif AA. Role of protein kinase C α in endothelin-1 stimulation of cytosolic phospholipase A2 and arachidonic acid release in cultured cat iris sphincter smooth muscle cells. *Biochim Biophys Acta*. 1998;1392(1):127-144.

47. Schramek H, Wang Y, Konieczkowski M, et al. Endothelin-1 stimulates cytosolic phospholipase A2 activity and gene expression in rat glomerular mesangial cells. *Kidney Int.* 1994;46(6):1644-1652.
48. Steffan M, Russell JA. Signal transduction in endothelin-induced contraction of rabbit pulmonary vein. *Pulm Pharmacol.* 1990;3(1):1-7.
49. Wright HM, Malik KU. Prostacyclin formation elicited by endothelin-1 in rat aorta is mediated via phospholipase D activation and not phospholipase C or A2. *Circ Res.* 1996;79(2):271-276.
50. Giustina A, Bossoni S, Cimino A, et al. Picotamide, a dual TXB synthetase inhibitor and TXB receptor antagonist, reduces exercise-induced albuminuria in microalbuminuric patients with NIDDM. *Diabetes.* 1993;42(1):178-182.
51. Giustina A, Ianniello P, Bossoni S, et al. Effect of long-term administration of picotamide on baseline and exercise-induced urinary albumin excretion in patients with type II diabetes mellitus and incipient nephropathy. *Clin Ther.* 1994;16(2):191-199.
52. Giustina A, Perini P, Desenzani P, et al. Long-term treatment with the dual antithromboxane agent picotamide decreases microalbuminuria in normotensive type 2 diabetic patients. *Diabetes.* 1998;47(3):423-430.
53. Amraoui F, Spijkers L, Hassani Lahsinoui H, et al. SFlt-1 elevates blood pressure by augmenting endothelin-1-mediated vasoconstriction in mice. *PLoS One.* 2014;9(3):e91897.

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