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Enhanced electrical field stimulated nitrenergic and purinergic vasoreactivity in distal versus proximal internal pudendal arteries

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Abstract

Background—The internal pudendal arteries (IPA) supply blood to the penis and are highly susceptible to vascular remodeling in rodent models of diabetes, hypertension, aging, and chronic kidney disease contributing to erectile dysfunction (ED). Interestingly, vascular remodeling primarily occurs in the distal and not in the proximal IPA suggesting that there are distinct local physiological signaling differences within the IPA.

Aim—To examine the role of purinergic signaling and electrical field stimulated (EFS) neurotransmitter release in regulation of proximal and distal IPA vascular tone.

Methods—Proximal and distal IPA were mounted in wire myographs and vascular responses to phenylephrine, acetylcholine, and DEA NONOate were measured. EFS mediated contraction and non-adrenergic non-cholinergic (NANC) relaxation was evaluated in the absence and presence of a nitric oxide synthase (NOS) antagonist. Purinergic agonist and NANC relaxation responses were assessed in the presence and absence of P2X1 and P2Y1 antagonists. Protein expression of P2X1 and P2Y1 receptors was measured by western blot.

Main Outcome Measures—Proximal and distal IPA contraction and relaxation was measured during increasing agonist administration and EFS in the presence and absence of antagonists.

Results—Proximal and distal IPA concentration responses curves to phenylephrine, acetylcholine and DEA NONOate were no different. Interestingly, distal IPA exhibited greater EFS-mediated contraction and NANC relaxation compared to proximal IPA. NOS inhibition completely inhibited distal IPA NANC relaxation but did not affect proximal IPA relaxation. P2X1 or P2Y1 receptor antagonism during NANC relaxation increased distal IPA relaxation but reduced

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proximal IPA relaxation. Combined P2X1 and P2Y1 receptor antagonism had no effect on proximal IPA relaxation but significantly increased distal IPA NANC relaxation.

Clinical Translation—Understanding neurovascular regulation of IPA vascular tone via nitrenergic and purinergic mechanisms may yield new therapeutic targets to improve IPA blood flow and treat vasculogenic ED.

Strengths & Limitations—This study is the first to illustrate the differences in mechanisms responsible for regulating vascular tone in the proximal and distal IPA. All presented findings are currently limited to ex vivo vascular function.

Conclusion—The regulation of vascular tone is different regionally in the IPA. The distal IPA is controlled through neurotransmitter mediated NO-dependent mechanisms and increased sensitivity to purinergic P2X1 and P2Y1 receptor inhibition.

Keywords

Erectile dysfunction; internal pudendal artery; electric field stimulation; vascular smooth muscle; purinergic signaling; neurotransmitter release

Introduction

Erectile function relies on complex signaling pathways that result in peripheral neurotransmitter mediated regulation of penile vascular tone. Disruption of one or more of these neurovascular signaling mechanisms may result in erectile dysfunction (ED), which is prevalent in over 18% of men greater than 20 years of age in the United States^[1]. ED is linked to aging, diabetes, hypertension and cardiovascular disease, and is often accompanied by symptoms of arteriosclerosis, fibrosis, and peripheral neuropathy which impede blood flow to the penis^[1,2]. Furthermore, ED in otherwise asymptomatic men is predictive of future cardiovascular disease, particularly in young men^[3,4].

A common goal of ED therapy is to increase blood flow to the penis. The internal pudendal arteries (IPA) are the primary arteries supplying blood to the penis and contribute to 70% of the total resistance in the penile vasculature^[5]. In humans and in the rat, the IPA originates from the internal iliac artery, runs along the lateral wall of the pelvis and terminates by branching into the dorsal and deep arteries of the penis^[6]. We and others have defined the proximal IPA as the segment from the internal iliac to the gluteal artery branch and the distal portion is from the gluteal artery to the bifurcation of the dorsal and deep penile arteries^[6,7]. Pathologic vascular remodeling in the distal IPA has been observed in rat models of aging, hypertension, and chronic kidney disease which also demonstrate ED^[6–8]. Maio and colleagues revealed extensive arterial wall calcification in the distal IPA from chronic kidney disease rats that was absent in the proximal IPA^[7]. The cause of the disproportionate pathological remodeling between the proximal and distal segments of the IPA is unknown. Distal IPA have been shown to have a thicker smooth muscle layer and a smaller lumen diameter compared to proximal IPA in rats^[7]. To our knowledge, the differences in vasoreactivity between the proximal and distal IPA have not been examined. Potential physiological differences in the signaling pathways of proximal and distal IPA may account for the structural variations observed in pathological conditions.

Vascular tone can be regulated by adrenergic, cholinergic, and non-adrenergic non-cholinergic (NANC) nerve terminals. Nitric oxide (NO) release via NANC nerve terminals is responsible for the initiation of penile vasodilation during an erection^[9]. Traditionally, NANC vasodilation is elicited by using electric field stimulation (EFS) in tissue incubated with antagonists of adrenergic neurotransmitter release and muscarinic receptors^[10,11]. However, the role of other neurotransmitters such as vasoactive intestinal peptide (VIP), calcitonin gene related peptide (CGRP), neuropeptide Y (NPY), and purinergic mediators (ADP, ATP) that can also be released from presynaptic nerve terminals are not accounted for.

ATP is a co-neurotransmitter simultaneously released from adrenergic, cholinergic, and NANC nerve terminals with norepinephrine, acetylcholine and NO that can mediate contractile and vasodilatory responses^[12]. Once released, ATP can be further degraded into ADP, cyclic adenosine monophosphate (cAMP), and adenosine through ectonuclease activity^[13]. ATP is known to bind purinergic P2X1 and P2Y1 receptors and ADP has a higher affinity for P2Y1 receptors^[12]. Stimulation of endothelial P2Y receptors is known to relax human corpus cavernosum^[14] while stimulation of P2X1 receptors in cavernous smooth muscle inhibits erections in diabetic rats^[15]. The role of purines in regulating vascular tone of the IPA has not been investigated. This study will characterize the adrenergic, NO-dependent, NO-independent and purinergic regulation of vascular tone within the proximal and distal segments of the IPA.

Methods

Animals

Adult (3–4 months old) male Sprague-Dawley rats (n= 36, 375–450 g, Charles River Laboratories, Morrisville, NC) were housed in pairs and provided with standard rodent chow and water *ad libitum* in a temperature controlled room ($22 \pm 2^\circ\text{C}$) with a 12 hour light/dark cycle. Animal experiments were authorized by the XXXXXXXXXX Animal Care and Use Committee under guidelines set forth by the National Institutes of Health (Guide for the Care and Use of Laboratory Animals, 8th edition) and the American Veterinary Medical Association. Animals were separated into two groups and used for myograph (n=28) and Western blot (n=8) experiments.

Sources of drugs and reagents

The following compounds were used for myograph studies and purchased from Sigma-Aldrich (St. Louis, MO, USA): (R)-(-)-phenylephrine hydrochloride (PE), acetylcholine chloride (ACh), adenosine 5' diphosphate sodium salt (ADP), potassium phosphate monobasic, potassium chloride, D-(+)-glucose, magnesium sulfate heptahydrate, sodium chloride, calcium chloride dehydrate, α,β -methylene adenosine 5'-triphosphate (α,β -MetATP), guanethidine, N $_{\omega}$ -Nitro-L-arginine methyl ester hydrochloride (L-NAME). DEA NONOate and NF449 were purchased from Cayman Chemicals (Ann Arbor, MI, USA), and MRS2500, 2-methylthioadenosine diphosphate trisodium salt (2-MeSADP), and tetrodotoxin citrate from Tocris (Bristol, United Kingdom). All compounds were dissolved in distilled water with the exception of atropine and DEA NONOate which were dissolved in 100% ethanol and further diluted using distilled water.

Vascular reactivity studies

Rats were anesthetized by intraperitoneal injection of ketamine/xylazine (90/10 mg/kg) and bilateral IPA were carefully excised and placed in ice-cold Krebs solution (130mM NaCl, 4.7mM KCl, 1.18mM KH_2PO_4 , 1.18mM MgSO_4 , 14.9mM NaHCO_3 , 5.6mM dextrose, and 1.56mM CaCl_2 in distilled water). IPA were classified as proximal (region extending from internal iliac artery to 1mm proximal of gluteal artery bifurcation) or distal (region 1mm distal of gluteal artery bifurcation to penile arteries bifurcation). Proximal and distal IPA (2mm rings) were mounted in wire myograph using 40 μm stainless steel wire (Danish Myograph Technology 620M, Aarhus, DK). Myograph chambers were filled with Krebs solution, bubbled with 95% O_2 /5% CO_2 , and maintained at 37°C. Optimal IPA resting tension was set using DMT normalization software followed by a 30 minute equilibration period. Smooth muscle and endothelial integrity were verified via contractile responses to 120mM high potassium solution (KCl), contraction via PE (10^{-6}M), and ACh (10^{-6}M) induced relaxation. Vessels were considered viable if they contracted over 10mN to KCl and relaxed 60% to ACh. Contractile responses were assessed by concentration response curves (CRC) to PE (10^{-9} – 10^{-5}M). Vessels were precontracted with PE (~80% of max contraction) and relaxation to ACh (10^{-9} – 10^{-5}M) and DEA-NONOate (10^{-9} – 10^{-5}M) was measured.

Proximal and distal IPA segments underwent electric field stimulation (EFS) to induce contraction using the following parameters: 0.5–32 Hz, 5 ms delay, 0.3 ms duration, 20 volts, and 10 second stimulations at 2 minute intervals. After a 30 minute incubation with guanethidine (10^{-6}M) and atropine (10^{-5}M), vessels were precontracted with PE and EFS mediated NANC relaxation was measured. NANC relaxation was also measured in the presence of tetrodotoxin (sodium channel antagonist, 10^{-5}M), L-NAME (NOS antagonist, 10^{-5}M), NF449 (P2X1 receptor antagonist, 10^{-5}M), MRS2500 (P2Y1 receptor antagonist, 10^{-6}M), or the combination of both NF449 and MRS2500.

Contraction and relaxation CRC to α,β -MetATP (10^{-9} – 10^{-5}M) were measured in the absence and presence of the P2X1 antagonist NF449. Vessels were precontracted with PE and relaxation to ADP (10^{-9} – 10^{-5}M) and 2-MeSADP (10^{-9} – 10^{-5}M) was measured in absence and presence of P2Y1 antagonist MRS2500. Antagonists were dosed and incubated as previously described.

Protein expression in distal and proximal IPA

Total protein from proximal and distal IPA samples (n=8/each) was extracted using RIPA buffer (150 mM NaCl, 1% Triton, 0.5% sodium deoxycholate, 0.1% SDS, and 50 mM Tris at a pH of 7.65). Protein concentration was determined through bicinchoninic acid protein assay and 20 μg of protein was loaded in 4–12% Bis-Tris SDS-polyacrylamide gel (Thermo Fisher, Waltham, MA, USA). Protein electrophoresis at 160 V for 60 minutes was conducted and proteins were transferred to nitrocellulose membrane. Membranes were incubated with rabbit polyclonal primary antibodies against either P2X1 (1:1,000) or P2Y1 (1:200) receptors (Alomone Labs, Jerusalem, Israel) and conjugated with goat anti-rabbit polyclonal horseradish peroxidase antibody (1:5,000; Thermo Fisher, Waltham, MA, USA). Bands were detected by chemiluminescence (Pierce ECL) and imaged with Bio-Rad ChemiDoc Touch (Bio-Rad Laboratories, Hercules, CA, USA). Membranes were stripped and β -actin

(1:1,000; Thermo Fisher) expression was evaluated to normalize P2X1 and P2Y1 receptor protein expression. Densitometry of the bands were analyzed using ImageJ software (NIH, Bethesda, MD, USA).

Data Analysis

Myograph data were analyzed using LabChart 8 software (AD Instruments: Colorado Springs, CO, USA) and are represented as mean $\pm$ standard error of the mean (SEM). Contractions were normalized as a percentage of the maximum KCl contraction. A nonlinear fit sigmoidal curve was applied to PE, ACh, and DEA NONOate concentration responses using GraphPad Prism 5.0 software (GraphPad Software, La Jolla, CA, USA). Relaxations were normalized as a percent of the PE precontracted value. Western blot P2X1 and P2Y1 receptor protein expression was normalized to β -actin expression. Two way ANOVAs and Bonferroni post hoc t-tests were used to assess significance between proximal and distal samples. Values with a $p < 0.05$ were considered statistically significant.

Results

Vasoreactivity of the proximal and distal IPA

The contractile responses to KCl and PE were similar in proximal and distal IPA (Figure 1A,B). Endothelium-dependent relaxation to ACh was unchanged in proximal and distal IPA (Figure 1C). Similarly, endothelium-independent relaxation to DEA NONOate caused both the proximal and distal IPA to relax 100% (Figure 1D).

EFS maximal contraction was 2-fold higher in the distal compared to the proximal IPA (Figure 2A,C,E). Additionally, NANC relaxation was greater in distal IPA (30%) relative to proximal IPA (16%; Figure 2B,D,F). To confirm that NANC relaxation was due to neurotransmitter release and not direct stimulation of the smooth muscle, NANC relaxation was performed in the presence of the neurotoxin, tetrodotoxin, which inhibits sodium channels and prevents presynaptic neurotransmitter vesicle release. Tetrodotoxin completely abolished relaxation in all proximal IPA segments (12/12) and in 80% of segments from the distal IPA (8/10; $n=4$ rats, data not shown) indicating that the NANC relaxation was due to presynaptic neurotransmitter release. To assess whether NANC relaxation was NO-dependent, EFS relaxation was performed in the presence of L-NAME. NOS inhibition did not change the NANC relaxation response in the proximal IPA (Figure 3A) but completely inhibited NANC relaxation in the distal IPA (Figure 3B).

Purinergic vasoreactivity in the proximal and distal IPA

The role of purinergic mediated contractile and relaxation responses were also examined. α,β -MetATP, a stable ATP analog and P2X1 receptor agonist, induced small contractions in both distal and proximal IPA (Figure 4A). In precontracted vessels, low concentrations of α,β -MetATP resulted in small relaxation responses, whereas higher concentrations caused contractions in the proximal and distal IPA (Figure 4B). ADP, a nonselective P2Y receptor agonist, induced greater relaxation in the proximal (Figure 4C). ADP is subjected to degradation into cAMP and adenosine by ectonucleotidase activity. To account for this, a stable ADP analog, 2-MeSADP, was used to assess P2Y1 receptors specifically.

Administration of 2-MesADP resulted in minimal relaxation in both IPA segments (Figure 4D).

The specificity of P2X1 and P2Y1 antagonists were evaluated by their ability to inhibit contraction or relaxation of known purinergic agonists. In arteries incubated with NF449, a P2X1 selective antagonist, the contractile responses to α,β -MetATP were eliminated in both the proximal and distal IPA (Figure 5A,B). MRS2500, a P2Y1 selective antagonist, did not affect ADP induced proximal IPA relaxation (Figure 5C) but inhibited ADP induced relaxation in the distal IPA (Figure 5D). MRS2500 did not impair 2-MeSADP mediated relaxation in proximal IPA (Figure 5E) or significantly inhibit relaxation in distal IPA (Figure 5F).

The role of purinergic neurotransmitter release during NANC relaxation was assessed by selectively antagonizing purinergic receptors using NF449 and MRS2500. P2X1 receptor antagonism with NF449 minimally decreased NANC relaxation in the proximal IPA (Figure 6A), but enhanced NANC relaxation in the distal IPA (Figure 6B). Antagonism of P2Y1 receptors via MRS2500 induced similar inhibition of proximal IPA NANC relaxation (Figure 6C) and had no impact on distal IPA NANC relaxation (Figure 6D). Antagonizing both P2X1 and P2Y1 receptors had no effect on the NANC response in the proximal IPA (Figure 6E). In contrast, P2X1 and P2Y1 receptor antagonism notably enhanced NANC relaxation 2-fold in the distal IPA (Figure 6F). Western blot analysis of P2X1 and P2Y1 receptor protein expression were similar in the proximal and distal IPA (Figure 7A,B).

Discussion

This study is the first to demonstrate that vascular tone is differentially regulated in the proximal and distal segments of the IPA. The distal IPA exhibits greater EFS mediated contraction and NANC relaxation responses compared to the proximal IPA. Furthermore, NANC mediated relaxation in the distal IPA is NO-dependent, whereas the proximal IPA is NO-independent. P2X1 and P2Y1 purinergic receptor antagonism further amplifies distal IPA NANC relaxation but in contrast does not affect proximal IPA NANC relaxation. These findings suggest that the distal IPA is under greater neurovascular regulation which may be essential for normal erectile physiological function.

The distal IPA have been shown to have an increased wall thickness and decreased lumen diameter compared to the proximal IPA indicating that distal IPA exhibit greater vascular resistance^[7]. Despite an increased medial smooth muscle layer, no notable differences in KCl or PE induced contractions were observed between proximal or distal IPA suggesting that ion channel and adrenergic receptor populations are similar. Increased vascular resistance correlates with an increase in shear stress, a known contributor to endothelial damage leading to vascular remodeling of smooth muscle in disease states such as hypertension, diabetes, and chronic kidney disease^[2,7,8,16]. Interestingly, in rats with chronic kidney disease, the distal IPA demonstrated greater smooth muscle remodeling and calcification that was otherwise absent in the proximal IPA or the penis^[7].

Sympathetic release of norepinephrine is known to induce detumescence through vasoconstriction of the cavernous sinusoids while NO released from NANC nerve terminals is the principal neurotransmitter responsible for initiating vasodilation in the penis^[9]. The distal IPA exhibit greater NO-mediated NANC relaxation and EFS mediated contraction suggesting greater innervation and/or neurotransmitter release than the proximal IPA. The significant increase in EFS mediated contraction warrants further evaluation in future studies. The neuronal origin of NANC relaxation mediators was confirmed using tetrodotoxin to inhibit synapse transmission and prevent NANC relaxation in the proximal and distal IPA. EFS mediated IPA contractions have been examined in rat models of hypogonadism and heart failure with contrasting data showing diminished contractions and increased peak contractions, respectively^[17,18]. In both studies, the segments of the IPA that were examined were not defined as distal or proximal IPA. The complete NO dependency in the distal IPA contrasts with data from mesenteric arteries in which NANC relaxation has been shown to be NO-independent^[19]. Conversely, NOS inhibition in mesenteric arteries undergoing EFS mediated relaxation has been shown to enhance^[20], but also impair relaxation^[21]. In regards to NO dependency, neurovascular regulation of the distal IPA more closely resembles the penis rather than other resistance vessels. Elucidating region specific IPA neurovascular regulation is crucial to understand how blood flow in different area may impact on pathological vascular remodeling in disease states.

Purinergic signaling has a dual role in regulating vascular tone. The biphasic response to α,β -MetATP seen in the IPA has also been observed in mesenteric arteries and is dependent on an intact endothelium^[22]. α,β -MetATP is also commonly used to desensitize P2X1 receptors in order to inhibit exogenous ATP induced contraction and receptor desensitization may be contributing to the overall contractile response that we are seeing in these cumulative CRCs^[23,24]. On the other hand, P2Y1 receptor stimulation with either 2-MeSADP or ADP vasodilates rat mesenteric arteries 80–100% yet only relaxes IPA by 5–15%^[25]. ADP elicited a 10% greater relaxation in the proximal IPA which could be attributed to increased ectonuclease mediated ADP hydrolysis into adenosine and subsequent activation of NO-independent adenosine receptors to regulate cAMP^[26]. The role of adenosine receptors in facilitating an erection is not yet completely understood. Adenosine receptors (A2A and A2B) have been identified to play a functional role in mouse IPA NO dependent vasodilation^[26]. Our findings show distal IPA, but not proximal IPA, relax through NANC mediated NO-dependent modulation of cGMP. While the mouse adenosine IPA study did not indicate what segment of the IPA was assessed, it would be of interest for future studies to see if the NO dependent relaxation to adenosine was only present in the distal segments of the IPA and not proximal. Although P2X1 receptors are predominantly expressed on smooth muscle, P2Y1 receptors are expressed on both endothelial and smooth muscle cells^[27] which may lead to biphasic results with P2Y1 antagonists as they can inhibit one or both receptor populations. Future studies will assess purinergic responses in denuded vessels to determine if the changes in vasoreactivity are smooth muscle or endothelial cell specific. Investigation into purinergic signaling is currently limited by the lack of commercially available P2X and P2Y subtype selective agonists and antagonists to assess physiological function of purinergic receptors.

Interestingly, dual purinergic receptor antagonism enhances NANC relaxation in the distal IPA. Although P2X1 and P2Y1 receptor protein expression was similar between proximal and distal IPA, receptor binding or activation following EFS remains unknown. ATP can be hydrolyzed and act on 7 different P2X or 8 different P2Y receptor subtypes^[28]. Inhibition of a single P2 receptor allows shunting of purinergic neurotransmitters to act on other P2 receptors. In human primary culture of umbilical endothelium and mammary smooth muscle cells, P2X4/P2Y11 receptor and P2Y2 receptors, respectively, are highly expressed^[27] and purines may be binding to these receptors during P2X1/P2Y1 antagonism. Activation of P2X4 and P2Y11 receptors facilitate the release of NO^[28] and dual activation of these receptors could mediate the synergistic increase in NANC relaxation observed in NO-dependent distal IPA. Further studies are required to fully characterize the P2X, P2Y and P2 receptor populations in the proximal and distal IPA. In human corpus cavernosum from men with vasculogenic ED, impaired relaxation to adenosine receptor A2B agonists were evident^[29]. Given that purinergic inhibition enhanced neurovascular responses in the distal IPA, further evaluation of purinergic signaling in animal models exhibiting ED or in human pathological cavernosal samples is warranted. Additionally, studies have shown that presynaptic purinergic receptors are localized on neurons in the enteric nervous system and the rat caudal tail artery which can modulate synaptic transmission via the release of norepinephrine^[30,31]. While the current study performed NANC relaxations in the presence of purinergic antagonists, it would be interesting in future studies to examine how the distal and proximal arteries responses to EFS contraction and NANC relaxation would be affected in the presence of purinergic agonists.

The IPA plays a vital role in regulating penile blood flow during an erectile response. This study is the first to compare regional differences in vascular reactivity in the IPA and evaluate the role of purinergic signaling. We have elucidated the distal and proximal IPA have unique physiology, are highly regulated by peripheral neurotransmitter release and respond differently to purinergic stimulation. With the advent of new selective purinergic agonists and antagonists, more discoveries will be made to gain insight into purinergic regulation of the IPA. Further investigation is warranted into purinergic signaling of the distal and proximal IPA in disease to confirm if purinergic drugs are a potential therapeutic for ED.

Abbreviations

IPA	internal pudendal arteries
EFS	electric field stimulation
ED	erectile dysfunction
NANC	non-adrenergic non-cholinergic
NO	nitric oxide
PE	(R)-(-)-phenylephrine hydrochloride
ACh	acetylcholine chloride

ADP	adenosine 5' diphosphate sodium salt
L-NAME	N _ω -Nitro-L-arginine methyl ester hydrochloride
ATP	adenosine triphosphate
α,β-MetATP	α,β-methylene adenosine 5'-triphosphate
2-MeSADP	2-methylthioadenosine diphosphate trisodium salt
KCl	high potassium solution
NOS	nitric oxide synthase
CRC	concentration response curve
SEM	standard error of the mean

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Figure 1. No differences in smooth muscle contractility, endothelial dependent relaxation, or endothelial independent relaxation
 (A) Contractile response to 120 mM KCl (n=16/segment). (B) PE concentration responses normalized to maximum KCl response fitted with nonlinear regression curve (n=10/segment). (C) Acetylcholine (n=10/segment) and (D) DEA NONOate (n=8/segment) concentration response curves in vessels precontracted using PE. For all values, mean $\pm$ SEM.

Figure 2. Increased EFS mediated contraction and NANC relaxation in distal IPA

Representative tracings of (A) proximal and (C) distal IPA EFS contraction compared to maximum KCl response. NANC relaxation representative tracings in (B) proximal and (D) distal IPA shown as change from PE precontraction. (E) EFS mediated contractions normalized to maximum KCl response (n=10/segment). (F) NANC relaxation expressed as percent decrease from PE precontraction (n=13/segment). For all values, mean $\pm$ SEM and *p<0.05 versus proximal IPA.

Figure 3. NO-dependent NANC relaxation in distal IPA

(A) NANC relaxation in the presence of L-NAME, a NOS antagonist, in proximal IPA (n=7) and (B) the distal IPA (n=7). All relaxation values are represented by percent decrease from PE precontraction. For all values, mean $\pm$ SEM and *p<0.05 versus proximal IPA.

Figure 4. Purinergic mediated contraction and relaxation of IPA

(A) α, β -MetATP induced contractions in proximal (n=9) and distal (n=9) IPA. Values normalized as percentage of maximum KCl response. (B) Relaxation via α, β -MetATP in proximal (n=9) and distal (n=9) precontracted IPA. Values represented as percentage relaxation from PE precontraction. (C) ADP induced relaxation as a percent decrease from PE precontraction (n=8/segment). (D) 2-MeSADP induced relaxation shown as a percent decrease from PE precontraction (n=8/segment). For all values, mean $\pm$ SEM and * $p < 0.05$ versus proximal IPA or antagonist.

Figure 5. Effectiveness of P2X1 and P2Y1 antagonists

(A) NF449 (10^{-5} M) eliminated α, β -MetATP induced contractions in proximal (n=6) and (B) distal IPA (n=6). (C) MRS2500 (10^{-6} M) did not impair ADP induced relaxation in proximal IPA (n=6) but (D) did decrease relaxation in distal IPA (n=6). (E) MRS2500 did not impair 2-MeSADP induced proximal IPA relaxation (n=8) or (F) reduce distal IPA relaxation to 2-MeSADP (n=8). For all values, mean $\pm$ SEM and * $p < 0.05$ versus antagonist.

Figure 6. Purinergic antagonism improves distal IPA NANC relaxation

(A) NANC relaxation with addition of selective P2X1 antagonist, NF449, in proximal and (B) distal IPA (n=8/segment). (C) NANC relaxation with addition of selective P2Y1 antagonist, MRS2500, in proximal and (D) distal IPA (n=8/segment). (E) Combined P2X1 and P2Y1 receptor antagonism in NANC relaxation using NF449 and MRS2500 in proximal and (F) distal IPA (n=8/segment). All values are represented as percent decrease from PE precontraction. For all values, mean $\pm$ SEM and *p<0.05 versus antagonist.

Figure 7. No differences in P2X1 and P2Y1 receptor protein expression

(A) P2X1 receptor expression in proximal and distal IPA normalized to β-actin. (B) P2Y1 receptor expression in proximal and distal IPA normalized to β-actin. For all values, n=8/group and mean ± SEM.