



ROLE OF Ca-ACTIVATED K CHANNELS AND Na,K-ATPase IN PROSTAGLANDIN E- AND E-INDUCED INHIBITION OF THE ADRENERGIC RESPONSE IN HUMAN VAS DEFERENS

Pascual Medina, Gloria Segarra, María Dolores Mauricio, José M. Vila,
Pascual Chuan, Salvador Lluch

► To cite this version:

Pascual Medina, Gloria Segarra, María Dolores Mauricio, José M. Vila, Pascual Chuan, et al..
ROLE OF Ca-ACTIVATED K CHANNELS AND Na,K-ATPase IN PROSTAGLANDIN E- AND
E-INDUCED INHIBITION OF THE ADRENERGIC RESPONSE IN HUMAN VAS DEFERENS.
Biochemical Pharmacology, 2011, 82 (1), pp.65. 10.1016/j.bcp.2011.03.021 . hal-00701260

HAL Id: hal-00701260

<https://hal.science/hal-00701260>

Submitted on 25 May 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

Title: ROLE OF Ca^{2+} -ACTIVATED K^{+} CHANNELS AND $\text{Na}^{+},\text{K}^{+}$ -ATPase IN PROSTAGLANDIN E_1 - AND E_2 -INDUCED INHIBITION OF THE ADRENERGIC RESPONSE IN HUMAN VAS DEFERENS

Authors: Pascual Medina, Gloria Segarra, María Dolores Mauricio, José M. Vila, Pascual Chuan, Salvador Lluch

PII: S0006-2952(11)00188-2
DOI: doi:10.1016/j.bcp.2011.03.021
Reference: BCP 10861

To appear in: *BCP*

Received date: 13-1-2011
Revised date: 21-3-2011
Accepted date: 22-3-2011

Please cite this article as: Medina P, Segarra G, Mauricio MD, Vila JM, Chuan P, Lluch S, ROLE OF Ca^{2+} -ACTIVATED K^{+} CHANNELS AND $\text{Na}^{+},\text{K}^{+}$ -ATPase IN PROSTAGLANDIN E_1 - AND E_2 -INDUCED INHIBITION OF THE ADRENERGIC RESPONSE IN HUMAN VAS DEFERENS, *Biochemical Pharmacology* (2008), doi:10.1016/j.bcp.2011.03.021

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



TITLE PAGE

ROLE OF Ca^{2+} -ACTIVATED K^{+} CHANNELS AND $\text{Na}^{+}, \text{K}^{+}$ -ATPase IN
PROSTAGLANDIN E_1 - AND E_2 -INDUCED INHIBITION OF THE ADRENERGIC
RESPONSE IN HUMAN VAS DEFERENS

Author's names and affiliations:

Pascual Medina^{a,b}, Gloria Segarra^{a,b}, María Dolores Mauricio^{a,b}, José M. Vila^{a,b}, Pascual Chuan^c, and Salvador Lluh^{a,b}

^aDepartamento de Fisiología, Facultad de Medicina y Odontología, Universidad de Valencia, Av. Blasco Ibáñez 15, 46010 Valencia, Spain, and ^bINCLIVA, Instituto Investigación Sanitaria, Hospital Clínico Universitario, Av. Blasco Ibáñez 17, 46010 Valencia, Spain, and ^cServicio de Urología Hospital Clínico Universitario, Av. Blasco Ibáñez 17, 46010 Valencia, Spain.

Corresponding author:

Pascual Medina
Departamento de Fisiología
Facultad de Medicina y Odontología
Av. Blasco Ibáñez, 15
46010 Valencia, Spain
Telephone number: +34 963864644
Fax number: +34 963864642
e-mail address: Pascual.Medina@uv.es

ABSTRACT

We studied the role of K^+ channels and Na^+,K^+ -ATPase in the presynaptic inhibitory effects of prostaglandin E_1 (PGE_1) and PGE_2 on the adrenergic responses of human vas deferens. Furthermore, we determined the effects of increasing extracellular K^+ concentrations ($[K^+]_o$) and inhibition of Na^+,K^+ -ATPase on neurogenic and norepinephrine -induced contractile responses. Ring segments of the epididymal part of the vas deferens were taken from 45 elective vasectomies and mounted in organ baths for isometric recording of tension. The neuromodulatory effects of PGEs were tested in the presence of K^+ channel blockers. PGE_1 and PGE_2 (10^{-8} - 10^{-6} M) induced inhibition of adrenergic contractions. The presence of tetraethylammonium (10^{-3} M), charybdotoxin (10^{-7} M), or iberiotoxin (10^{-7} M), prevented the inhibitory effects of PGE_1 and PGE_2 on the adrenergic contraction. Both glibenclamide (10^{-5} M) and apamin (10^{-6} M) failed to antagonize PGE_1 and PGE_2 effects. Raising the $[K^+]_o$ from **15.8** mM to **25.8** mM caused inhibition of the neurogenic contractions. Ouabain at a concentration insufficient to alter the resting tension (10^{-6} M) increased contractions induced by electrical stimulation but did not alter the contractions to norepinephrine. The inhibition of neurogenic responses induced PGE_1 , PGE_2 and increased extracellular concentration of K^+ was almost completely prevented by ouabain (10^{-6} M). The results demonstrate that PGE_1 and PGE_2 inhibit adrenergic responses by a prejunctional mechanism that involves the activation of large-conductance Ca^{2+} -activated K^+ channels and Na^+,K^+ -ATPase.

Keywords: Electrical field stimulation, Human vas deferens, Norepinephrine, Potassium channels, Prostaglandins, Smooth muscle.

1. INTRODUCTION

Prostaglandins are a family of biologically lipid acids synthesized by cyclooxygenase from a common precursor, arachidonic acid. Previous studies have indicated that prostaglandin E₁ (PGE₁) and PGE₂, and other prostanoids, influence adrenergic neurotransmission in the vas deferens from several species [1-4]. In human vas deferens, the motor innervation is mainly noradrenergic [5,6] and PGE₁ and PGE₂ have been reported to inhibit neurogenic contractions by decreasing the release of norepinephrine from sympathetic nerve endings [7]. **The mechanisms whereby PGE₁ and PGE₂ modify neurotransmitter release have received much attention but remain poorly understood. Prostaglandins of the E series inhibit transmitter release by an action on stimulus-secretion coupling and more specifically on the availability of Ca²⁺ for the release mechanism [8].**

K⁺ channels modulate the adrenergic contractile responses in human vas deferens [9] and several agents have been shown to inhibit neurotransmitter release from nerve endings through a mechanism that involves the opening of prejunctional K⁺ channels, membrane hyperpolarization and reduction in Ca²⁺ influx via voltage-activated Ca²⁺ channels [10,11]. In vas deferens, it has been demonstrated that K⁺ channels are involved in the prejunctional inhibitory effects of atrial natriuretic factor [12], α_2 -adrenoceptor agonists [13] and sildenafil, an inhibitor of phosphodiesterase 5 [14]. Activation of K⁺ channels stimulates cellular K⁺ efflux [15]. In vascular smooth muscle, it has been demonstrated that an increased extracellular K⁺ concentration ([K⁺]_o) evokes rapid hyperpolarization and relaxation [16] by stimulating an ouabain-sensitive Na⁺,K⁺-ATPase [17]. In rat vas deferens, increased extracellular K⁺ concentrations lower than those necessary to induce contraction of the smooth muscle inhibit electrically-induced

contractions [18]. A role for increased $[K^+]_o$ and Na^+,K^+ -ATPase in modulation of adrenergic neurotransmission in the human vas deferens has not yet been investigated. Therefore, the present study was designed to examine the effects of increasing $[K^+]_o$ and Na^+, K^+ -ATPase on adrenergic contractions of human vas deferens and the contribution of K^+ channels and Na^+,K^+ -ATPase to the inhibitory effects of PGE_1 and PGE_2 on adrenergic neurotransmission.

2. MATERIAL AND METHODS

Segments (15-20 mm long) of the epididymal part of the vas deferens were taken from **45 healthy men without previous history of psychiatric or physical illness, medication use, or substance abuse (mean age, 39, range 30–44 years)** who were sterilized by elective vasectomy. The study was approved by the **Human** Ethics Committee of our institution and informed consent was obtained from each subject before the study. The specimens were placed in chilled isotonic NaCl, and were divided into ring preparations 3-4 mm long.

2.1 Isolated vas deferens preparation

Ring preparations were suspended between two L-shaped stainless steel pins. One pin was fixed to the organ bath wall while the other was connected to a strain gauge (Grass FT03, Grass Instruments Division Astromed, Inc., West Warwick, RI, U.S.A.). Changes in isometric force were recorded by use of Chart v3.4/s software and a MacLab/8e data acquisition system (ADInstruments, East Sussex, U.K.). Each preparation was set up in a 4-ml bath containing modified Krebs-Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6; KH₂PO₄ 1.2; MgCl₂·6H₂O, 1.2; CaCl₂, 2.5; NaHCO₃, 25; glucose, 11.1; and disodium EDTA, 0.01. The solution was equilibrated with 95% O₂ and 5% CO₂. The preparations were allowed to equilibrate for 1-h and during this time tension was adjusted to a final tension of 19.6 mN.

Electrical field stimulation was provided by a Grass S88 stimulator (Grass Instruments Division Astromed, Inc., West Warwick, RI, U.S.A.) via two platinum electrodes positioned on each side and parallel to the axis of the ring. Single square wave pulses (0.25 ms pulse duration, 20 Hz, at a supramaximal voltage of 20 Vcm⁻¹) were used. The

train duration was 5 s and the stimulation interval 180 s. The stimulation parameters used elicit contractile responses that are abolished by tetrodotoxin (10^{-6} M) or prazosin (10^{-6} M) [19].

2.2 Experimental procedure.

To study the neuromodulatory action of prostaglandins, when electrically induced phasic contractions were stable (after 15-20 minutes), PGE₁ or PGE₂ (10^{-8} – 10^{-6} M) were added cumulatively to the preparations and the effects of electrical field stimulation were recorded. To examine the role of K⁺ channel activation in the effects of PGE₁ and PGE₂ on electrical field stimulation induced contractions, concentration–response curves to PGE₁ and PGE₂ were established in the presence of one of the following inhibitors: TEA (10^{-3} M), a nonspecific K⁺ channel blocker [20], glibenclamide (10^{-5} M), a selective blocker of ATP-sensitive K⁺ (K_{ATP}) channels [21], charybdotoxin (10^{-7} M), an inhibitor of Ca²⁺-activated K⁺ (K_{Ca}) channels of both large-conductance (K_{Ca}1.1) [22] and intermediate-conductance (K_{Ca}3.1) [23], iberiotoxin (10^{-7} M), an inhibitor of K_{Ca}1.1 channels [24], or apamin (10^{-6} M), an inhibitor of K_{Ca} of small-conductance (K_{Ca}2) channels [25].

Concentration-response curves to norepinephrine were obtained in a cumulative manner in the absence (control) and in the presence of PGE₁ (10^{-6} M) or PGE₂ (10^{-6} M) from separate preparations.

K⁺ channels activation stimulates K⁺ efflux [15]. As increases in the [K⁺]_o evoke rapid vascular smooth muscle relaxation and hyperpolarization [16], we studied the adrenergic responses in the presence of increased [K⁺]_o (**10.8-25.8 mM**) to check the possibility that increasing [K⁺]_o may inhibit adrenergic contractions. At the end of each series, the physiological concentration of K⁺ was restored by washing out with Krebs-

Henseleit solution and electrical stimulation was repeated to confirm the reversibility of the effects of high K^+ concentrations on neurogenic response.

To determine whether Na^+, K^+ -ATPase activity is involved in the adrenergic responses of the human vas deferens, the contractions to electrical field stimulation and norepinephrine were recorded before and 15 min after the addition of ouabain (10^{-7} – 10^{-6} M), a Na^+, K^+ -ATPase inhibitor.

In another group of experiments, the influence of Na^+, K^+ -ATPase on the PGE_1 -, PGE_2 - or increasing K^+ concentrations -induced inhibition of contractions evoked by electrical field stimulation was examined by exposing vas deferens rings to ouabain (10^{-6} M) for 15 min before the addition of PGE_1 (10^{-8} – 3×10^{-6} M), PGE_2 (10^{-8} – 3×10^{-6} M) or $[K^+]_o$ (**10.8-25.8 mM**) and the responses to electrical stimulation were recorded.

2.3 Drugs

The following drugs were used: norepinephrine hydrochloride, prazosin hydrochloride, tetrodotoxin, tetraethylammonium bromide (TEA), charybdotoxin, iberiotoxin, apamin, glibenclamide and ouabain (Sigma Chemical Co. St. Louis, MO, USA). All drugs were dissolved in Krebs solution, except for glibenclamide and ouabain which were dissolved initially in dimethyl sulphoxide and further diluted in Krebs solution to the proper final concentration. Stock solutions of the drugs were freshly prepared every day, and kept on ice throughout the experiment.

2.4 Data analysis

All values are expressed as means \pm S.E.M. Contractions are reported as absolute values (mN) or as percentages of control responses. pD_2 values (negative logarithm of the molar concentration at which half-maximum contraction occurs) were determined from

individual concentration-response curves by non-linear regression analysis. The responses obtained in each subject were averaged to yield a single value. Differences between untreated and treated groups were assessed by one-way analysis of variance (ANOVA), and then Bonferroni's test was performed. Statistical significance was accepted at $P < 0.05$.

Accepted Manuscript

3. RESULTS

Electrical field stimulation (EFS) induced phasic contraction in segments of the vas deferens which was abolished by tetrodotoxin (10^{-6} M) and prazosin (10^{-6} M), thus indicating that the smooth muscle contraction was due to the release of norepinephrine from adrenergic nerves acting on α_1 adrenoceptors (results not shown).

Figure 1A shows representative tracings of the effects of PGE₁ (10^{-8} - 10^{-6} M) on EFS-evoked contractions of the vas deferens rings in the absence (control) and in the presence of K⁺ channel blockers and Figure 1B and C summarizes the results from all the experiments. PGE₁ induced concentration-dependent inhibition of EFS-evoked contractions with a pD₂ value of 7.35 ± 0.10 (Figure 1B and C). Apamin (10^{-6} M) and glibenclamide (10^{-5} M) did not modify the inhibitory effect of PGE₁ on neurogenic contractions whereas charybdotoxin (10^{-7} M), TEA (10^{-3} M) and iberiotoxin (10^{-7} M) reduced significantly the inhibitory effects of PGE₁. The effects of PGE₂ on neurogenic contractions and the inhibition of these effects by K⁺ channel blockers were similar to those observed with PGE₁ (Figure 2).

Figure 3 (A and B) shows that the increase in [K⁺]_o in the Krebs-Henseleit solution caused concentration-dependent inhibition of the EFS-induced contractions with a maximum inhibitory effect of 96%. The inhibition was reversible after washout of the excess of K⁺.

Ouabain (10^{-7} - 10^{-6} M) had no significant effect on the basal tone of the preparations but increased contractile responses to EFS (Figure 3C and D).

Ouabain (10^{-6} M) counteracted the inhibitory effects of PGE₁, PGE₂ and increasing [K⁺]_o on EFS-induced contractions (Figure 4).

Cumulative addition of norepinephrine (10^{-6} – 3×10^{-4} M) induced repetitive phasic, concentration-dependent contractions with a pD_2 value of 5.01 ± 0.10 . Pretreatment with PGE_1 (10^{-6} M) or PGE_2 (10^{-6} M) did not affect the contractions induced by norepinephrine (Table 1). Moreover, pretreatment with either ouabain (10^{-6} M) or increasing $[K^+]_o$ to 25.8 mM were without effect on norepinephrine-induced contractions (Figure 5 and Table 1).

4. DISCUSSION

The present study demonstrates a role for K^+ channels and Na^+,K^+ -ATPase in the prejunctional inhibitory effects of PGE_1 and PGE_2 on adrenergic responses of human vas deferens. It has been demonstrated that PGE_1 and PGE_2 selectively depress neurogenic contractions in human vas deferens without modifying the contractions to norepinephrine [7]. The lack of effect of PGE_1 and PGE_2 on norepinephrine-induced contractions suggests that postjunctional inhibitory mechanisms are not involved in the neuromodulatory action of these prostaglandins. The inhibitory effect on neurogenic contractions has been attributed to decreased norepinephrine release from nerve endings in the human vas deferens [7].

The mechanisms whereby PGE_1 and PGE_2 induce inhibition of neurotransmitter release remain poorly understood. Several agents could inhibit neurotransmitter release from nerve endings in vas deferens through a mechanism that involves activation of K^+ channels. **In human vas deferens, charybdotoxin-sensitive, iberiotoxin-insensitive, K^+ channels modulate the adrenergic contractile response by interfering with Ca^{2+} entry through dihydropyridine Ca^{2+} channels [9].** Furthermore, it has been shown that K^+ channels are involved in the prejunctional inhibitory effects of atrial natriuretic factor in the rabbit isolated vas deferens [12] and in the α_2 -adrenoceptor-mediated inhibition in rat vas deferens [13]. In the human vas deferens, sildenafil, an inhibitor of phosphodiesterase 5, inhibits adrenergic contractions through activation of presynaptic $K_{Ca1.1}$ channels [14].

Glibenclamide has been shown to block K_{ATP} channels [21] with an IC_{50} in the micromolar range for smooth muscle preparations [26]. In rat vas deferens, the inhibitory action of calcitonin-gene-related peptide (CGRP) on neurotransmission was

at least in part mediated by the activation of K_{ATP} channels in sympathetic nerve terminals as shown by the prevention of the neuromodulatory effects of CGRP in the presence of glibenclamide 10^{-5} M [27]. Our results in human vas deferens demonstrate that glibenclamide at concentration as high as 10^{-5} M did not modify the inhibition induced by PGEs on adrenergic responses. These results rule out the involvement of K_{ATP} channels activation in the inhibitory effects of PGEs on neurogenic contractions of the human vas deferens.

On the other hand, we tested the effects of TEA, a nonspecific K^+ channel blocker [20] on the neuromodulatory effects of PGE_1 and PGE_2 . It is accepted that blockade with 10^{-3} M TEA is sufficient to almost fully inhibit $K_{Ca1.1}$ channels [28]. In our experiments millimolar concentrations of TEA prevented the inhibitory effects of PGE_1 and PGE_2 on neurogenic contractions, suggesting a role of K_{Ca} channels in this effect. To further characterize the member of K_{Ca} channel group involved in the neuromodulatory effects of PGEs we used more selective blockers of K_{Ca} channels. Apamin has proved to be extremely valuable as a highly specific blocker for which the only known receptors are the K_{Ca} channels of small conductance (K_{Ca2}) [29]. In our experiments, apamin 10^{-6} M, a concentration expected to block completely all subtypes of K_{Ca2} channels, failed to alter the inhibition caused by PGE_1 and PGE_2 on neurogenic contractions in human vas deferens. These data indicate that K_{Ca2} channels are not involved in the neuromodulatory effects of PGE_1 and PGE_2 .

Charybdotoxin, a blocker of both $K_{Ca1.1}$ and $K_{Ca3.1}$ channels [22,23], prevented the inhibitory effects of PGE_1 and PGE_2 on sympathetic contractions of the human vas deferens. These results demonstrate that the K^+ channels involved are sensitive to charybdotoxin. Therefore, we tested the effect of iberiotoxin, a selective blocker of $K_{Ca1.1}$ channels [24], on the effects of PGE_1 and PGE_2 on adrenergic contractions. The

marked effect of iberiotoxin in the present study provides evidence for $K_{Ca1.1}$ channels as a significant component of PGE_1 and PGE_2 inhibitory effects on neurogenic contractions of the human vas deferens.

A previous study in rat vas deferens demonstrated that increasing $[K^+]_o$, at concentrations lower than those necessary to induce contraction of the smooth muscle, completely inhibited the electrically-induced contractions [18], an effect attributed to a desensitization of the rat vas deferens to electrically-induced contractions. Studies of vascular smooth muscle showed that increased $[K^+]_o$ in the range 6-16 mM produce hyperpolarization and vasodilatation by stimulating an ouabain-sensitive Na^+,K^+ -ATPase [17]. In guinea-pig vas deferens relatively high concentrations of ouabain, an inhibitor of Na^+,K^+ -ATPase, is reported to cause a gradual increase in the norepinephrine release from the peripheral adrenergic neurons [30] and an increase in the sensitivity to norepinephrine [31]. Particularly for the vas deferens, species is thought to play an important role in Na^+,K^+ -ATPase activity and function [31]. However, the effects of inhibition of Na^+,K^+ -ATPase on adrenergic responses of human vas deferens have not been investigated. As the resting tension of human vas deferens was not changed by ouabain in our experiments, we suggest that Na^+,K^+ -ATPase is not normally active in the relaxed vas deferens smooth muscle. Furthermore, ouabain had no effect on norepinephrine-mediated contractions at concentration of 10^{-6} M, but increased neurogenic contractions. These results indicate that the enhanced neurogenic contractions induced by ouabain involves the inhibition of Na^+,K^+ -ATPase activity of the adrenergic nerve endings.

Increasing $[K^+]_o$ over the range that inhibits electrical field stimulation-induced adrenergic contractions should also stimulate the Na^+,K^+ -ATPase by increasing the amount of extracellular K^+ that binds to the pump [32]. In human vas deferens, ouabain

blocks K^+ -induced inhibition of adrenergic contractions, indicating that increasing $[K^+]_o$ stimulates the Na^+,K^+ -ATPase in the range that inhibits adrenergic contractions.

Because Na^+,K^+ -ATPase activity is ultimately responsible for most of the resting membrane potential, the ability of **increased** extracellular K^+ to regulate adrenergic contractions could in part be due to its effect on Na^+,K^+ -ATPase activity. **There are two effects of the pump on membrane potential: (i) an electrogenic effect, due to the $3Na^+/2K^+$ exchange (this is the smaller, but more rapid effect) and (ii) by maintaining the transmembrane K^+ gradient the membrane potential is created (this is the more powerful, but slower effect).** Our results suggest that in the inhibitory effects of K^+ on the adrenergic contractions of human vas deferens only the first effect is relevant because in our experimental conditions it is not possible to increase the transmembrane K^+ gradient by increasing $[K^+]_o$.

However, involvement of other mechanisms/pathways cannot be excluded.

Recently, it has been demonstrated the proximity and functional interaction between the $KCa1.1$ channel and Na^+,K^+ -ATPase [33]. This interplay is suggested to depend on the proper microenvironment. Previous studies demonstrated that inhibition of Na^+,K^+ -ATPase and subsequent accumulation of intracellular Na^+ decreases $KCa1.1$ current [33,34]. Another possibility is that inhibition of Na^+,K^+ -ATPase by ouabain leads to an accumulation of Na^+ close to the microdomains harboring $KCa1.1$ channels, which inhibits the channel function.

Furthermore, ouabain blocked the inhibitory effects of PGE_1 and PGE_2 on sympathetic contractions in human vas deferens, suggesting that activation of Na^+,K^+ -ATPase plays an important role in the presynaptic inhibition of the norepinephrine release induced by these prostaglandins. **Consistent with our data, an acute stimulatory effect of PGE_1**

and PGE₂ on Na⁺,K⁺-ATPase activity was observed in rabbit renal proximal tubule cells and cortical collecting tubule of the nephron [35,36].

In rat vas deferens it has been demonstrated that PGE₂ is released from the epithelium in response to ATP stimulation and it is responsible for mediating the membrane hyperpolarization and inhibition of contraction of smooth muscle cells by activating cAMP-dependent K⁺ channels [37]. However, our results show that PGE₂ inhibited neurogenic contractions but did not exert inhibitory effects on norepinephrine-induced contractions, indicating that PGE₂ acts directly on sympathetic nerve endings.

An important function of the vas deferens is the transport of spermatozoa from the epididymis for inclusion in the semen. Since α_1 -adrenoceptors activation by norepinephrine is required for normal contraction of the vas deferens and consequent sperm ejaculation [38], it seems likely that increased formation of PGE₂ in physiological and pathological conditions such as inflammation [39], may cause aperistalsis of the adrenergically innervated vas deferens **by activating K_{Ca}1.1 channels and Na⁺,K⁺-ATPase** and impairment of male fertility. **Although the present studies provide evidence that activation of presynaptic K_{Ca}1.1 channels inhibits adrenergic contractions of vas deferens, future studies are necessary to elucidate the potential therapeutic modification of vas deferens motility, via pharmacologic intervention of these channels.**

The findings of the present study support the view that the presynaptic inhibitory effect of PGE₁ and PGE₂ is mediated via activation of large-conductance K_{Ca} channels.

Increasing [K⁺]_o induces reversible inhibition of adrenergic vas deferens contractions.

These inhibitory effects on neurogenic contractions of human vas deferens are mediated by activation of Na⁺,K⁺-ATPase.

ACKNOWLEDGEMENTS

This work was supported by the Ministerio de Ciencia e Innovación and Conselleria de Sanidad de la Generalitat Valenciana.

Accepted Manuscript

REFERENCES

- [1] Hedqvist P, von Euler US. Prostaglandin-induced neurotransmission failure in the field-stimulated, isolated vas deferens. *Neuropharmacology* 1972; 11:177-87.
- [2] Illes P, Ronai A, Knoll J. Inhibition of neuromuscular transmission by prostaglandin E1 in the circular muscle of the guinea-pig vas deferens. *Med Biol* 1976; 54:400-5.
- [3] Miller MR, Johnson FC, Poyser NL. Endogenous prostaglandin formation in the field-stimulated guinea-pig vas deferens: comparison of the inhibitory effects of indomethacin and NS-398. *Prostaglandins Leukot Essent Fatty Acids* 1999; 61:363-8.
- [4] McKay AM, Poyser NL. A comparison of the effects of exogenous and endogenous prostaglandins on fast and slow contractions of field-stimulated guinea-pig vas deferens. *Br J Pharmacol* 1995; 116:2679-84.
- [5] Birmingham AT. The human isolated vas deferens: its response to electrical stimulation and to drugs. *Br J Pharmacol* 1968; 34:692P-3P.
- [6] Hedlund H, Andersson KE, Larsson B. Effect of drugs interacting with adrenoceptors and muscarinic receptors in the epididymal and prostatic parts of the human isolated vas deferens. *J Auton Pharmacol* 1985; 5:261-70.
- [7] Holmquist F, Hedlund H, Andersson KE. Pre- and postjunctional effects of some prostanoids in human isolated vas deferens. *Am J Physiol* 1991; 260:R792-R797.
- [8] Malik KU, Sehic E. Prostaglandins and the release of the adrenergic transmitter. *Ann N Y Acad Sci* 1990; 604:222-36.

- [9] Medina P, Segarra G, Mauricio MD, Vila JM, Chuan P, Lluch S. Modulation of adrenergic responses of human vas deferens by K^+ channel inhibitors. *Urology* 2010; 76:1518-12.
- [10] North RA, Williams JT, Surprenant A, Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. *Proc Natl Acad Sci U S A* 1987; 84:5487-91.
- [11] Miller RJ. Receptor-mediated regulation of calcium channels and neurotransmitter release. *FASEB J* 1990; 4:3291-9.
- [12] Kanwal S, Trachte GJ. Potassium channel inhibitors attenuate neuromodulatory effects of atrial natriuretic factor in the rabbit isolated vas deferens. *J Pharmacol Exp Ther* 1994; 268:117-23.
- [13] Docherty JR, Brady G. Prejunctional actions of K^+ channel blockers in rat vas deferens. *Eur J Pharmacol* 1995; 287:287-93.
- [14] Medina P, Segarra G, Torondel B, Chuan P, Domenech C, Vila JM, Lluch S. Inhibition of neuroeffector transmission in human vas deferens by sildenafil. *Br J Pharmacol* 2000; 131:871-4.
- [15] Gordon JL, Martin W. Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of ^{86}Rb efflux from isolated endothelial cells. *Br J Pharmacol* 1983; 79:531-41.
- [16] Edwards FR, Hirst GD. Inward rectification in submucosal arterioles of guinea-pig ileum. *J Physiol* 1988; 404:437-54.
- [17] Webb RC, Bohr DF. Potassium-induced relaxation as an indicator of Na^+/K^+ ATPase activity in vascular smooth muscle. *Blood Vessels* 1978; 15:198-207.
- [18] Banchelli G, Raimondi L, Ghelardini C, Pirisino R, Bertini V, De MA, Lucchesini F. Benzylamine-related compounds stimulate rat vas deferens neurotransmission

and potentiate memory in the mouse acting as potassium channel blockers.

Pharmacol Res 2000; 41:151-62.

- [19] Medina P, Segarra G, Chuan P, Domenech C, Vila JM, Aldasoro M, Lluch S.

Vasopressin receptors involved in adrenergic neurotransmission in the circular muscle of the human vas deferens. Eur J Pharmacol 1998; 355:41-9.

- [20] Benham CD, Bolton TB, Lang RJ, Takewaki T. The mechanism of action of Ba^{2+} and TEA on single Ca^{2+} -activated K^{+} -channels in arterial and intestinal smooth muscle cell membranes. Pflugers Arch 1985; 403:120-7.

- [21] Sturgess NC, Ashford ML, Cook DL, Hales CN. The sulphonylurea receptor may be an ATP-sensitive potassium channel. Lancet 1985; 2:474-5.

- [22] Miller C, Moczydlowski E, Latorre R, Phillips M. Charybdotoxin, a protein inhibitor of single Ca^{2+} -activated K^{+} channels from mammalian skeletal muscle. Nature 1985; 313:316-8.

- [23] Brugnara C, Armsby CC, De FL, Crest M, Euclaire MF, Alper SL. Ca^{2+} -activated K^{+} channels of human and rabbit erythrocytes display distinctive patterns of inhibition by venom peptide toxins. J Membr Biol 1995; 147:71-82.

- [24] Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ, Garcia ML. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. J Biol Chem 1990; 265:11083-90.

- [25] Blatz AL, Magleby KL. Single apamin-blocked Ca-activated K^{+} channels of small conductance in cultured rat skeletal muscle. Nature 1986; 323:718-20.

- [26] Winquist RJ, Heaney LA, Wallace AA, Baskin EP, Stein RB, Garcia ML, Kaczorowski GJ. Glyburide blocks the relaxation response to BRL 34915

- (cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. *J Pharmacol Exp Ther* 1989; 248:149-56.
- [27] Huang Y, Lau CW, Ho IH. Involvement of ATP-sensitive K^+ channels in the inhibitory effect of calcitonin gene-related peptide on neurotransmission in rat vas deferens. *Eur J Pharmacol* 1997; 327:209-14.
- [28] Kaczorowski GJ, Knaus HG, Leonard RJ, McManus OB, Garcia ML. High-conductance calcium-activated potassium channels; structure, pharmacology, and function. *J Bioenerg Biomembr* 1996; 28:255-67.
- [29] Stocker M. Ca^{2+} -activated K^+ channels: molecular determinants and function of the SK family. *Nat Rev Neurosci* 2004; 5:758-70.
- [30] Nakazato Y, Ohga A, Onoda Y. The effect of ouabain on noradrenaline output from peripheral adrenergic neurones of isolated guinea-pig vas deferens. *J Physiol* 1978; 278:45-54.
- [31] Urquilla PR, Westfall DP, Goto K, Fleming WW. The effects of ouabain and alterations in potassium concentration on the sensitivity to drugs and the membrane potential of the smooth muscle of the guinea-pig and rat vas deferens. *J Pharmacol Exp Ther* 1978; 207:347-55.
- [32] Glynn IM. Sodium and potassium movements in human red cells. *J Physiol* 1956; 134:278-310.
- [33] Tajima N, Itokazu Y, Korpi ER, Somerharju P, Kakela R. Activity of BK_{Ca} channel is modulated by membrane cholesterol content and association with Na^+/K^+ -ATPase in human melanoma IGR39 cells. *J Biol Chem* 2011; 286:5624-38.

- [34] Liang GH, Kim MY, Park S, Kim JA, Choi S, Suh SH. Intracellular Na^+ modulates large conductance Ca^{2+} -activated K^+ currents in human umbilical vein endothelial cells. *Pflugers Arch* 2008; 457:67-75.
- [35] Scherzer P, Wald H, Rubinger D, Popovtzer MM. Indomethacin and sodium retention in the rat: role of inhibition of prostaglandin E_2 synthesis. *Clin Sci (Lond)* 1992; 83:307-11.
- [36] Herman MB, Rajkhowa T, Cutuli F, Springate JE, Taub ML. Regulation of Renal Proximal Tubule Na,K-ATPase by Prostaglandins. *Am J Physiol Renal Physiol* 2010.
- [37] Ruan YC, Wang Z, Du JY, Zuo WL, Guo JH, Zhang J, Wu ZL, Wong HY, Chung YW, Chan HC, Zhou WL. Regulation of smooth muscle contractility by the epithelium in rat vas deferens: role of ATP-induced release of PGE_2 . *J Physiol* 2008; 586:4843-57.
- [38] Sanbe A, Tanaka Y, Fujiwara Y, Tsumura H, Yamauchi J, Cotecchia S, Koike K, Tsujimoto G, Tanoue A. Alpha1-adrenoceptors are required for normal male sexual function. *Br J Pharmacol* 2007; 152:332-40.
- [39] Legler DF, Bruckner M, Uetz-von AE, Krause P. Prostaglandin E_2 at new glance: novel insights in functional diversity offer therapeutic chances. *Int J Biochem Cell Biol* 2010; 42:198-201.

FIGURE LEGENDS

Figure 1. A) Representative tracings showing the inhibitory effect of prostaglandin E_1 on electrical field stimulation (EFS)-evoked contractions of human vas deferens rings in the absence and in the presence of K^+ channel blockers. B and C summarize the results from all the experiments. Results were calculated as the percentage of the contractile response elicited by EFS (0.25 ms pulse duration, 20 Hz, at a supramaximal voltage of 20 V cm^{-1} , train duration 5 s) before the addition of prostaglandin E_1 . Values are presented as the mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group.

Figure 2. Graphical representation of the inhibition of EFS-induced contractions by increasing concentrations of prostaglandin E_2 in the absence (control) and in the presence of K^+ channel blockers. Results were calculated as the percentage of the contractile response elicited by EFS before the addition of prostaglandin E_2 . Values are presented as the mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group.

Figure 3. A) Representative tracing and B) bar graph showing the inhibitory effects of increasing extracellular K^+ concentration ($[K^+]_o$) from 5.8 to 25.8 mM on electrical field stimulation (EFS)-induced contractions. After wash out (W) with fresh Krebs-Henseleit solution ($[K^+]_o = 5.8 \text{ mM}$), the mean amplitude of contractions induced by EFS was reverted to initial conditions. C) Representative tracing and D) bar graph showing the contractile responses to EFS in the absence (control) and in the presence of ouabain ($10^{-7} - 10^{-6} \text{ M}$). Results in B) and D) were calculated as the

percentage of the contractile response elicited by EFS before the addition of K^+ or ouabain. Values are mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group. $*P<0.05$ versus control.

Figure 4. Inhibition of electrical field stimulation (EFS)-induced contractions by prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2) and increasing extracellular K^+ concentration ($[K^+]_o$) from 10.8 to 25.8 mM in the absence (control) and in the presence of ouabain (10^{-6} M). Results were calculated as the percentage of the contractile response elicited by EFS before the addition of PGE_1 , PGE_2 or K^+ . Values are mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group.

Figure 5. A) Recordings illustrating the contractile effects of norepinephrine in the absence (control, extracellular K^+ concentration $[K^+]_o = 5.8$ mM) and in the presence of $[K^+]_o$ of 25.8 mM or ouabain (10^{-6} M). Concentration-response curves for norepinephrine in the absence (control, $[K^+]_o = 5.8$ mM) and in the presence of B) $[K^+]_o$ of 25.8 mM, and C) in the presence of ouabain (10^{-6} M). Values are mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group. $*P<0.05$ versus control.

TITLE PAGE

ROLE OF Ca^{2+} -ACTIVATED K^+ CHANNELS AND Na^+, K^+ -ATPase IN
PROSTAGLANDIN E_1 - AND E_2 -INDUCED INHIBITION OF THE ADRENERGIC
RESPONSE IN HUMAN VAS DEFERENS

Author's names and affiliations:

Pascual Medina^{a,b}, Gloria Segarra^{a,b}, María Dolores Mauricio^{a,b}, José M. Vila^{a,b}, Pascual
Chuan^c, and Salvador Lluch^{a,b}

^aDepartamento de Fisiología, Facultad de Medicina y Odontología, Universidad de
Valencia, Av. Blasco Ibáñez 15, 46010 Valencia, Spain, and ^bINCLIVA, Instituto
Investigación Sanitaria, Hospital Clínico Universitario, Av. Blasco Ibáñez 17, 46010
Valencia, Spain, and ^cServicio de Urología Hospital Clínico Universitario, Av. Blasco
Ibáñez 17, 46010 Valencia, Spain.

Corresponding author:

Pascual Medina
Departamento de Fisiología
Facultad de Medicina y Odontología
Av. Blasco Ibáñez, 15
46010 Valencia, Spain
Telephone number: +34 963864644
Fax number: +34 963864642
e-mail address: Pascual.Medina@uv.es

ABSTRACT

We studied the role of K^+ channels and Na^+,K^+ -ATPase in the presynaptic inhibitory effects of prostaglandin E_1 (PGE_1) and PGE_2 on the adrenergic responses of human vas deferens. Furthermore, we determined the effects of increasing extracellular K^+ concentrations ($[K^+]_o$) and inhibition of Na^+,K^+ -ATPase on neurogenic and norepinephrine -induced contractile responses. Ring segments of the epididymal part of the vas deferens were taken from 45 elective vasectomies and mounted in organ baths for isometric recording of tension. The neuromodulatory effects of PGEs were tested in the presence of K^+ channel blockers. PGE_1 and PGE_2 (10^{-8} - 10^{-6} M) induced inhibition of adrenergic contractions. The presence of tetraethylammonium (10^{-3} M), charybdotoxin (10^{-7} M), or iberiotoxin (10^{-7} M), prevented the inhibitory effects of PGE_1 and PGE_2 on the adrenergic contraction. Both glibenclamide (10^{-5} M) and apamin (10^{-6} M) failed to antagonize PGE_1 and PGE_2 effects. Raising the $[K^+]_o$ from 15.8 mM to 25.8 mM caused inhibition of the neurogenic contractions. Ouabain at a concentration insufficient to alter the resting tension (10^{-6} M) increased contractions induced by electrical stimulation but did not alter the contractions to norepinephrine. The inhibition of neurogenic responses induced PGE_1 , PGE_2 and increased extracellular concentration of K^+ was almost completely prevented by ouabain (10^{-6} M). The results demonstrate that PGE_1 and PGE_2 inhibit adrenergic responses by a prejunctional mechanism that involves the activation of large-conductance Ca^{2+} -activated K^+ channels and Na^+,K^+ -ATPase.

Keywords: Electrical field stimulation, Human vas deferens, Norepinephrine, Potassium channels, Prostaglandins, Smooth muscle.

1. INTRODUCTION

Prostaglandins are a family of biologically lipid acids synthesized by cyclooxygenase from a common precursor, arachidonic acid. Previous studies have indicated that prostaglandin E₁ (PGE₁) and PGE₂, and other prostanoids, influence adrenergic neurotransmission in the vas deferens from several species [1-4]. In human vas deferens, the motor innervation is mainly noradrenergic [5,6] and PGE₁ and PGE₂ have been reported to inhibit neurogenic contractions by decreasing the release of norepinephrine from sympathetic nerve endings [7]. The mechanisms whereby PGE₁ and PGE₂ modify neurotransmitter release have received much attention but remain poorly understood. Prostaglandins of the E series inhibit transmitter release by an action on stimulus-secretion coupling and more specifically on the availability of Ca²⁺ for the release mechanism [8].

K⁺ channels modulate the adrenergic contractile responses in human vas deferens [9] and several agents have been shown to inhibit neurotransmitter release from nerve endings through a mechanism that involves the opening of prejunctional K⁺ channels, membrane hyperpolarization and reduction in Ca²⁺ influx via voltage-activated Ca²⁺ channels [10,11]. In vas deferens, it has been demonstrated that K⁺ channels are involved in the prejunctional inhibitory effects of atrial natriuretic factor [12], α_2 -adrenoceptor agonists [13] and sildenafil, an inhibitor of phosphodiesterase 5 [14]. Activation of K⁺ channels stimulates cellular K⁺ efflux [15]. In vascular smooth muscle, it has been demonstrated that an increased extracellular K⁺ concentration ([K⁺]_o) evokes rapid hyperpolarization and relaxation [16] by stimulating an ouabain-sensitive Na⁺,K⁺-ATPase [17]. In rat vas deferens, increased extracellular K⁺ concentrations lower than those necessary to induce contraction of the smooth muscle inhibit electrically-induced

1 contractions [18]. A role for increased $[K^+]_o$ and Na^+, K^+ -ATPase in modulation of
2 adrenergic neurotransmission in the human vas deferens has not yet been investigated.
3
4 Therefore, the present study was designed to examine the effects of increasing $[K^+]_o$ and
5
6 Na^+, K^+ -ATPase on adrenergic contractions of human vas deferens and the contribution
7
8 of K^+ channels and Na^+, K^+ -ATPase to the inhibitory effects of PGE_1 and PGE_2 on
9
10 adrenergic neurotransmission.
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65

2. MATERIAL AND METHODS

Segments (15-20 mm long) of the epididymal part of the vas deferens were taken from 45 healthy men without previous history of psychiatric or physical illness, medication use, or substance abuse (mean age, 39, range 30–44 years) who were sterilized by elective vasectomy. The study was approved by the Human Ethics Committee of our institution and informed consent was obtained from each subject before the study. The specimens were placed in chilled isotonic NaCl, and were divided into ring preparations 3-4 mm long.

2.1 Isolated vas deferens preparation

Ring preparations were suspended between two L-shaped stainless steel pins. One pin was fixed to the organ bath wall while the other was connected to a strain gauge (Grass FT03, Grass Instruments Division Astromed, Inc., West Warwick, RI, U.S.A.). Changes in isometric force were recorded by use of Chart v3.4/s software and a MacLab/8e data acquisition system (ADInstruments, East Sussex, U.K.). Each preparation was set up in a 4-ml bath containing modified Krebs-Henseleit solution of the following millimolar composition: NaCl, 115; KCl, 4.6; KH_2PO_4 1.2; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 1.2; CaCl_2 , 2.5; NaHCO_3 , 25; glucose, 11.1; and disodium EDTA, 0.01. The solution was equilibrated with 95% O_2 and 5% CO_2 . The preparations were allowed to equilibrate for 1-h and during this time tension was adjusted to a final tension of 19.6 mN.

Electrical field stimulation was provided by a Grass S88 stimulator (Grass Instruments Division Astromed, Inc., West Warwick, RI, U.S.A.) via two platinum electrodes positioned on each side and parallel to the axis of the ring. Single square wave pulses (0.25 ms pulse duration, 20 Hz, at a supramaximal voltage of 20 V cm^{-1}) were used. The

train duration was 5 s and the stimulation interval 180 s. The stimulation parameters used elicit contractile responses that are abolished by tetrodotoxin (10^{-6} M) or prazosin (10^{-6} M) [19].

2.2 Experimental procedure.

To study the neuromodulatory action of prostaglandins, when electrically induced phasic contractions were stable (after 15-20 minutes), PGE₁ or PGE₂ (10^{-8} – 10^{-6} M) were added cumulatively to the preparations and the effects of electrical field stimulation were recorded. To examine the role of K⁺ channel activation in the effects of PGE₁ and PGE₂ on electrical field stimulation induced contractions, concentration–response curves to PGE₁ and PGE₂ were established in the presence of one of the following inhibitors: TEA (10^{-3} M), a nonspecific K⁺ channel blocker [20], glibenclamide (10^{-5} M), a selective blocker of ATP-sensitive K⁺ (K_{ATP}) channels [21], charybdotoxin (10^{-7} M), an inhibitor of Ca²⁺-activated K⁺ (K_{Ca}) channels of both large-conductance (K_{Ca}1.1) [22] and intermediate-conductance (K_{Ca}3.1) [23], iberiotoxin (10^{-7} M), an inhibitor of K_{Ca}1.1 channels [24], or apamin (10^{-6} M), an inhibitor of K_{Ca} of small-conductance (K_{Ca}2) channels [25].

Concentration-response curves to norepinephrine were obtained in a cumulative manner in the absence (control) and in the presence of PGE₁ (10^{-6} M) or PGE₂ (10^{-6} M) from separate preparations.

K⁺ channels activation stimulates K⁺ efflux [15]. As increases in the [K⁺]_o evoke rapid vascular smooth muscle relaxation and hyperpolarization [16], we studied the adrenergic responses in the presence of increased [K⁺]_o (10.8-25.8 mM) to check the possibility that increasing [K⁺]_o may inhibit adrenergic contractions. At the end of each series, the physiological concentration of K⁺ was restored by washing out with Krebs-

Henseleit solution and electrical stimulation was repeated to confirm the reversibility of the effects of high K^+ concentrations on neurogenic response.

To determine whether Na^+, K^+ -ATPase activity is involved in the adrenergic responses of the human vas deferens, the contractions to electrical field stimulation and norepinephrine were recorded before and 15 min after the addition of ouabain (10^{-7} – 10^{-6} M), a Na^+, K^+ -ATPase inhibitor.

In another group of experiments, the influence of Na^+, K^+ -ATPase on the PGE_1 -, PGE_2 - or increasing K^+ concentrations -induced inhibition of contractions evoked by electrical field stimulation was examined by exposing vas deferens rings to ouabain (10^{-6} M) for 15 min before the addition of PGE_1 (10^{-8} – 3×10^{-6} M), PGE_2 (10^{-8} – 3×10^{-6} M) or $[K^+]_o$ (10.8–25.8 mM) and the responses to electrical stimulation were recorded.

2.3 Drugs

The following drugs were used: norepinephrine hydrochloride, prazosin hydrochloride, tetrodotoxin, tetraethylammonium bromide (TEA), charybdotoxin, iberiotoxin, apamin, glibenclamide and ouabain (Sigma Chemical Co. St. Louis, MO, USA). All drugs were dissolved in Krebs solution, except for glibenclamide and ouabain which were dissolved initially in dimethyl sulphoxide and further diluted in Krebs solution to the proper final concentration. Stock solutions of the drugs were freshly prepared every day, and kept on ice throughout the experiment.

2.4 Data analysis

All values are expressed as means \pm S.E.M. Contractions are reported as absolute values (mN) or as percentages of control responses. pD_2 values (negative logarithm of the molar concentration at which half-maximum contraction occurs) were determined from

individual concentration-response curves by non-linear regression analysis. The responses obtained in each subject were averaged to yield a single value. Differences between untreated and treated groups were assessed by one-way analysis of variance (ANOVA), and then Bonferroni's test was performed. Statistical significance was accepted at $P < 0.05$.

3. RESULTS

Electrical field stimulation (EFS) induced phasic contraction in segments of the vas deferens which was abolished by tetrodotoxin (10^{-6} M) and prazosin (10^{-6} M), thus indicating that the smooth muscle contraction was due to the release of norepinephrine from adrenergic nerves acting on α_1 adrenoceptors (results not shown).

Figure 1A shows representative tracings of the effects of PGE_1 (10^{-8} - 10^{-6} M) on EFS-evoked contractions of the vas deferens rings in the absence (control) and in the presence of K^+ channel blockers and Figure 1B and C summarizes the results from all the experiments. PGE_1 induced concentration-dependent inhibition of EFS-evoked contractions with a pD_2 value of 7.35 ± 0.10 (Figure 1B and C). Apamin (10^{-6} M) and glibenclamide (10^{-5} M) did not modify the inhibitory effect of PGE_1 on neurogenic contractions whereas charybdotoxin (10^{-7} M), TEA (10^{-3} M) and iberiotoxin (10^{-7} M) reduced significantly the inhibitory effects of PGE_1 . The effects of PGE_2 on neurogenic contractions and the inhibition of these effects by K^+ channel blockers were similar to those observed with PGE_1 (Figure 2).

Figure 3 (A and B) shows that the increase in $[\text{K}^+]_o$ in the Krebs-Henseleit solution caused concentration-dependent inhibition of the EFS-induced contractions with a maximum inhibitory effect of 96%. The inhibition was reversible after washout of the excess of K^+ .

Ouabain (10^{-7} - 10^{-6} M) had no significant effect on the basal tone of the preparations but increased contractile responses to EFS (Figure 3C and D). Ouabain (10^{-6} M) counteracted the inhibitory effects of PGE_1 , PGE_2 and increasing $[\text{K}^+]_o$ on EFS-induced contractions (Figure 4).

Cumulative addition of norepinephrine (10^{-6} – 3×10^{-4} M) induced repetitive phasic, concentration-dependent contractions with a pD_2 value of 5.01 ± 0.10 . Pretreatment with PGE_1 (10^{-6} M) or PGE_2 (10^{-6} M) did not affect the contractions induced by norepinephrine (Table 1). Moreover, pretreatment with either ouabain (10^{-6} M) or increasing $[K^+]_o$ to 25.8 mM were without effect on norepinephrine-induced contractions (Figure 5 and Table 1).

4. DISCUSSION

The present study demonstrates a role for K^+ channels and Na^+,K^+ -ATPase in the prejunctional inhibitory effects of PGE_1 and PGE_2 on adrenergic responses of human vas deferens. It has been demonstrated that PGE_1 and PGE_2 selectively depress neurogenic contractions in human vas deferens without modifying the contractions to norepinephrine [7]. The lack of effect of PGE_1 and PGE_2 on norepinephrine-induced contractions suggests that postjunctional inhibitory mechanisms are not involved in the neuromodulatory action of these prostaglandins. The inhibitory effect on neurogenic contractions has been attributed to decreased norepinephrine release from nerve endings in the human vas deferens [7].

The mechanisms whereby PGE_1 and PGE_2 induce inhibition of neurotransmitter release remain poorly understood. Several agents could inhibit neurotransmitter release from nerve endings in vas deferens through a mechanism that involves activation of K^+ channels. In human vas deferens, charybdotoxin-sensitive, iberiotoxin-insensitive, K^+ channels modulate the adrenergic contractile response by interfering with Ca^{2+} entry through dihydropyridine Ca^{2+} channels [9]. Furthermore, it has been shown that K^+ channels are involved in the prejunctional inhibitory effects of atrial natriuretic factor in the rabbit isolated vas deferens [12] and in the α_2 -adrenoceptor-mediated inhibition in rat vas deferens [13]. In the human vas deferens, sildenafil, an inhibitor of phosphodiesterase 5, inhibits adrenergic contractions through activation of presynaptic $K_{Ca1.1}$ channels [14].

Glibenclamide has been shown to block K_{ATP} channels [21] with an IC_{50} in the micromolar range for smooth muscle preparations [26]. In rat vas deferens, the inhibitory action of calcitonin-gene-related peptide (CGRP) on neurotransmission was

at least in part mediated by the activation of K_{ATP} channels in sympathetic nerve terminals as shown by the prevention of the neuromodulatory effects of CGRP in the presence of glibenclamide 10^{-5} M [27]. Our results in human vas deferens demonstrate that glibenclamide at concentration as high as 10^{-5} M did not modify the inhibition induced by PGEs on adrenergic responses. These results rule out the involvement of K_{ATP} channels activation in the inhibitory effects of PGEs on neurogenic contractions of the human vas deferens.

On the other hand, we tested the effects of TEA, a nonspecific K^+ channel blocker [20] on the neuromodulatory effects of PGE_1 and PGE_2 . It is accepted that blockade with 10^{-3} M TEA is sufficient to almost fully inhibit $K_{Ca1.1}$ channels [28]. In our experiments millimolar concentrations of TEA prevented the inhibitory effects of PGE_1 and PGE_2 on neurogenic contractions, suggesting a role of K_{Ca} channels in this effect.

To further characterize the member of K_{Ca} channel group involved in the neuromodulatory effects of PGEs we used more selective blockers of K_{Ca} channels.

Apamin has proved to be extremely valuable as a highly specific blocker for which the only known receptors are the K_{Ca} channels of small conductance (K_{Ca2}) [29]. In our experiments, apamin 10^{-6} M, a concentration expected to block completely all subtypes of K_{Ca2} channels, failed to alter the inhibition caused by PGE_1 and PGE_2 on neurogenic contractions in human vas deferens. These data indicate that K_{Ca2} channels are not involved in the neuromodulatory effects of PGE_1 and PGE_2 .

Charybdotoxin, a blocker of both $K_{Ca1.1}$ and $K_{Ca3.1}$ channels [22,23], prevented the inhibitory effects of PGE_1 and PGE_2 on sympathetic contractions of the human vas deferens. These results demonstrate that the K^+ channels involved are sensitive to charybdotoxin. Therefore, we tested the effect of iberiotoxin, a selective blocker of $K_{Ca1.1}$ channels [24], on the effects of PGE_1 and PGE_2 on adrenergic contractions. The

marked effect of iberiotoxin in the present study provides evidence for $K_{Ca1.1}$ channels as a significant component of PGE_1 and PGE_2 inhibitory effects on neurogenic contractions of the human vas deferens.

A previous study in rat vas deferens demonstrated that increasing $[K^+]_o$, at concentrations lower than those necessary to induce contraction of the smooth muscle, completely inhibited the electrically-induced contractions [18], an effect attributed to a desensitization of the rat vas deferens to electrically-induced contractions. Studies of vascular smooth muscle showed that increased $[K^+]_o$ in the range 6-16 mM produce hyperpolarization and vasodilatation by stimulating an ouabain-sensitive Na^+, K^+ -ATPase [17]. In guinea-pig vas deferens relatively high concentrations of ouabain, an inhibitor of Na^+, K^+ -ATPase, is reported to cause a gradual increase in the norepinephrine release from the peripheral adrenergic neurons [30] and an increase in the sensitivity to norepinephrine [31]. Particularly for the vas deferens, species is thought to play an important role in Na^+, K^+ -ATPase activity and function [31]. However, the effects of inhibition of Na^+, K^+ -ATPase on adrenergic responses of human vas deferens have not been investigated. As the resting tension of human vas deferens was not changed by ouabain in our experiments, we suggest that Na^+, K^+ -ATPase is not normally active in the relaxed vas deferens smooth muscle. Furthermore, ouabain had no effect on norepinephrine-mediated contractions at concentration of 10^{-6} M, but increased neurogenic contractions. These results indicate that the enhanced neurogenic contractions induced by ouabain involves the inhibition of Na^+, K^+ -ATPase activity of the adrenergic nerve endings.

Increasing $[K^+]_o$ over the range that inhibits electrical field stimulation-induced adrenergic contractions should also stimulate the Na^+, K^+ -ATPase by increasing the amount of extracellular K^+ that binds to the pump [32]. In human vas deferens, ouabain

blocks K^+ -induced inhibition of adrenergic contractions, indicating that increasing $[K^+]_o$ stimulates the Na^+,K^+ -ATPase in the range that inhibits adrenergic contractions.

Because Na^+,K^+ -ATPase activity is ultimately responsible for most of the resting membrane potential, the ability of increased extracellular K^+ to regulate adrenergic contractions could in part be due to its effect on Na^+,K^+ -ATPase activity. There are two effects of the pump on membrane potential: (i) an electrogenic effect, due to the $3Na^+/2K^+$ exchange (this is the smaller, but more rapid effect) and (ii) by maintaining the transmembrane K^+ gradient the membrane potential is created (this is the more powerful, but slower effect). Our results suggest that in the inhibitory effects of K^+ on the adrenergic contractions of human *vas deferens* only the first effect is relevant because in our experimental conditions it is not possible to increase the transmembrane K^+ gradient by increasing $[K^+]_o$.

However, involvement of other mechanisms/pathways cannot be excluded. Recently, it has been demonstrated the proximity and functional interaction between the $KCa1.1$ channel and Na^+,K^+ -ATPase [33]. This interplay is suggested to depend on the proper microenvironment. Previous studies demonstrated that inhibition of Na^+,K^+ -ATPase and subsequent accumulation of intracellular Na^+ decreases $KCa1.1$ current [33,34]. Another possibility is that inhibition of Na^+,K^+ -ATPase by ouabain leads to an accumulation of Na^+ close to the microdomains harboring $KCa1.1$ channels, which inhibits the channel function.

Furthermore, ouabain blocked the inhibitory effects of PGE_1 and PGE_2 on sympathetic contractions in human *vas deferens*, suggesting that activation of Na^+,K^+ -ATPase plays an important role in the presynaptic inhibition of the norepinephrine release induced by these prostaglandins. Consistent with our data, an acute stimulatory effect of PGE_1 and

1 PGE₂ on Na⁺,K⁺-ATPase activity was observed in rabbit renal proximal tubule cells and
2 cortical collecting tubule of the nephron [35,36].
3

4 In rat vas deferens it has been demonstrated that PGE₂ is released from the epithelium in
5 response to ATP stimulation and it is responsible for mediating the membrane
6
7 hyperpolarization and inhibition of contraction of smooth muscle cells by activating
8
9 cAMP-dependent K⁺ channels [37]. However, our results show that PGE₂ inhibited
10
11 neurogenic contractions but did not exert inhibitory effects on norepinephrine-induced
12
13 contractions, indicating that PGE₂ acts directly on sympathetic nerve endings.
14
15
16

17 An important function of the vas deferens is the transport of spermatozoa from the
18
19 epididymis for inclusion in the semen. Since α_1 -adrenoceptors activation by
20
21 norepinephrine is required for normal contraction of the vas deferens and consequent
22
23 sperm ejaculation [38], it seems likely that increased formation of PGE₂ in
24
25 physiological and pathological conditions such as inflammation [39], may cause
26
27 aperistalsis of the adrenergically innervated vas deferens by activating K_{Ca}1.1 channels
28
29 and Na⁺,K⁺-ATPase and impairment of male fertility. Although the present studies
30
31 provide evidence that activation of presynaptic K_{Ca}1.1 channels inhibits adrenergic
32
33 contractions of vas deferens, future studies are necessary to elucidate the potential
34
35 therapeutic modification of vas deferens motility, via pharmacologic intervention of
36
37 these channels.
38
39
40
41
42
43
44
45

46 The findings of the present study support the view that the presynaptic inhibitory effect
47
48 of PGE₁ and PGE₂ is mediated via activation of large-conductance K_{Ca} channels.
49
50

51 Increasing [K⁺]_o induces reversible inhibition of adrenergic vas deferens contractions.
52

53 These inhibitory effects on neurogenic contractions of human vas deferens are mediated
54
55 by activation of Na⁺,K⁺-ATPase.
56
57
58
59
60
61
62
63
64
65

ACKNOWLEDGEMENTS

This work was supported by the Ministerio de Ciencia e Innovación and Conselleria de Sanidad de la Generalitat Valenciana.

REFERENCES

- [1] Hedqvist P, von Euler US. Prostaglandin-induced neurotransmission failure in the field-stimulated, isolated vas deferens. *Neuropharmacology* 1972; 11:177-87.
- [2] Illes P, Ronai A, Knoll J. Inhibition of neuromuscular transmission by prostaglandin E1 in the circular muscle of the guinea-pig vas deferens. *Med Biol* 1976; 54:400-5.
- [3] Miller MR, Johnson FC, Poyser NL. Endogenous prostaglandin formation in the field-stimulated guinea-pig vas deferens: comparison of the inhibitory effects of indomethacin and NS-398. *Prostaglandins Leukot Essent Fatty Acids* 1999; 61:363-8.
- [4] McKay AM, Poyser NL. A comparison of the effects of exogenous and endogenous prostaglandins on fast and slow contractions of field-stimulated guinea-pig vas deferens. *Br J Pharmacol* 1995; 116:2679-84.
- [5] Birmingham AT. The human isolated vas deferens: its response to electrical stimulation and to drugs. *Br J Pharmacol* 1968; 34:692P-3P.
- [6] Hedlund H, Andersson KE, Larsson B. Effect of drugs interacting with adrenoceptors and muscarinic receptors in the epididymal and prostatic parts of the human isolated vas deferens. *J Auton Pharmacol* 1985; 5:261-70.
- [7] Holmquist F, Hedlund H, Andersson KE. Pre- and postjunctional effects of some prostanoids in human isolated vas deferens. *Am J Physiol* 1991; 260:R792-R797.
- [8] Malik KU, Sehic E. Prostaglandins and the release of the adrenergic transmitter. *Ann N Y Acad Sci* 1990; 604:222-36.

- [9] Medina P, Segarra G, Mauricio MD, Vila JM, Chuan P, Lluch S. Modulation of adrenergic responses of human vas deferens by K^+ channel inhibitors. *Urology* 2010; 76:1518-12.
- [10] North RA, Williams JT, Surprenant A, Christie MJ. Mu and delta receptors belong to a family of receptors that are coupled to potassium channels. *Proc Natl Acad Sci U S A* 1987; 84:5487-91.
- [11] Miller RJ. Receptor-mediated regulation of calcium channels and neurotransmitter release. *FASEB J* 1990; 4:3291-9.
- [12] Kanwal S, Trachte GJ. Potassium channel inhibitors attenuate neuromodulatory effects of atrial natriuretic factor in the rabbit isolated vas deferens. *J Pharmacol Exp Ther* 1994; 268:117-23.
- [13] Docherty JR, Brady G. Prejunctional actions of K^+ channel blockers in rat vas deferens. *Eur J Pharmacol* 1995; 287:287-93.
- [14] Medina P, Segarra G, Torondel B, Chuan P, Domenech C, Vila JM, Lluch S. Inhibition of neuroeffector transmission in human vas deferens by sildenafil. *Br J Pharmacol* 2000; 131:871-4.
- [15] Gordon JL, Martin W. Endothelium-dependent relaxation of the pig aorta: relationship to stimulation of ^{86}Rb efflux from isolated endothelial cells. *Br J Pharmacol* 1983; 79:531-41.
- [16] Edwards FR, Hirst GD. Inward rectification in submucosal arterioles of guinea-pig ileum. *J Physiol* 1988; 404:437-54.
- [17] Webb RC, Bohr DF. Potassium-induced relaxation as an indicator of Na^+/K^+ ATPase activity in vascular smooth muscle. *Blood Vessels* 1978; 15:198-207.
- [18] Banchelli G, Raimondi L, Ghelardini C, Pirisino R, Bertini V, De MA, Lucchesini F. Benzylamine-related compounds stimulate rat vas deferens neurotransmission

and potentiate memory in the mouse acting as potassium channel blockers.

Pharmacol Res 2000; 41:151-62.

- [19] Medina P, Segarra G, Chuan P, Domenech C, Vila JM, Aldasoro M, Lluch S. Vasopressin receptors involved in adrenergic neurotransmission in the circular muscle of the human vas deferens. Eur J Pharmacol 1998; 355:41-9.
- [20] Benham CD, Bolton TB, Lang RJ, Takewaki T. The mechanism of action of Ba^{2+} and TEA on single Ca^{2+} -activated K^{+} -channels in arterial and intestinal smooth muscle cell membranes. Pflugers Arch 1985; 403:120-7.
- [21] Sturgess NC, Ashford ML, Cook DL, Hales CN. The sulphonylurea receptor may be an ATP-sensitive potassium channel. Lancet 1985; 2:474-5.
- [22] Miller C, Moczydlowski E, Latorre R, Phillips M. Charybdotoxin, a protein inhibitor of single Ca^{2+} -activated K^{+} channels from mammalian skeletal muscle. Nature 1985; 313:316-8.
- [23] Brugnara C, Armsby CC, De FL, Crest M, Euclaire MF, Alper SL. Ca^{2+} -activated K^{+} channels of human and rabbit erythrocytes display distinctive patterns of inhibition by venom peptide toxins. J Membr Biol 1995; 147:71-82.
- [24] Galvez A, Gimenez-Gallego G, Reuben JP, Roy-Contancin L, Feigenbaum P, Kaczorowski GJ, Garcia ML. Purification and characterization of a unique, potent, peptidyl probe for the high conductance calcium-activated potassium channel from venom of the scorpion *Buthus tamulus*. J Biol Chem 1990; 265:11083-90.
- [25] Blatz AL, Magleby KL. Single apamin-blocked Ca-activated K^{+} channels of small conductance in cultured rat skeletal muscle. Nature 1986; 323:718-20.
- [26] Winquist RJ, Heaney LA, Wallace AA, Baskin EP, Stein RB, Garcia ML, Kaczorowski GJ. Glyburide blocks the relaxation response to BRL 34915

(cromakalim), minoxidil sulfate and diazoxide in vascular smooth muscle. *J Pharmacol Exp Ther* 1989; 248:149-56.

- [27] Huang Y, Lau CW, Ho IH. Involvement of ATP-sensitive K^+ channels in the inhibitory effect of calcitonin gene-related peptide on neurotransmission in rat vas deferens. *Eur J Pharmacol* 1997; 327:209-14.
- [28] Kaczorowski GJ, Knaus HG, Leonard RJ, McManus OB, Garcia ML. High-conductance calcium-activated potassium channels; structure, pharmacology, and function. *J Bioenerg Biomembr* 1996; 28:255-67.
- [29] Stocker M. Ca^{2+} -activated K^+ channels: molecular determinants and function of the SK family. *Nat Rev Neurosci* 2004; 5:758-70.
- [30] Nakazato Y, Ohga A, Onoda Y. The effect of ouabain on noradrenaline output from peripheral adrenergic neurones of isolated guinea-pig vas deferens. *J Physiol* 1978; 278:45-54.
- [31] Urquilla PR, Westfall DP, Goto K, Fleming WW. The effects of ouabain and alterations in potassium concentration on the sensitivity to drugs and the membrane potential of the smooth muscle of the guinea-pig and rat vas deferens. *J Pharmacol Exp Ther* 1978; 207:347-55.
- [32] Glynn IM. Sodium and potassium movements in human red cells. *J Physiol* 1956; 134:278-310.
- [33] Tajima N, Itokazu Y, Korpi ER, Somerharju P, Kakela R. Activity of BK_{Ca} channel is modulated by membrane cholesterol content and association with Na^+/K^+ -ATPase in human melanoma IGR39 cells. *J Biol Chem* 2011; 286:5624-38.

- [34] Liang GH, Kim MY, Park S, Kim JA, Choi S, Suh SH. Intracellular Na^+ modulates large conductance Ca^{2+} -activated K^+ currents in human umbilical vein endothelial cells. *Pflugers Arch* 2008; 457:67-75.
- [35] Scherzer P, Wald H, Rubinger D, Popovtzer MM. Indomethacin and sodium retention in the rat: role of inhibition of prostaglandin E_2 synthesis. *Clin Sci (Lond)* 1992; 83:307-11.
- [36] Herman MB, Rajkhowa T, Cutuli F, Springate JE, Taub ML. Regulation of Renal Proximal Tubule Na,K-ATPase by Prostaglandins. *Am J Physiol Renal Physiol* 2010.
- [37] Ruan YC, Wang Z, Du JY, Zuo WL, Guo JH, Zhang J, Wu ZL, Wong HY, Chung YW, Chan HC, Zhou WL. Regulation of smooth muscle contractility by the epithelium in rat vas deferens: role of ATP-induced release of PGE_2 . *J Physiol* 2008; 586:4843-57.
- [38] Sanbe A, Tanaka Y, Fujiwara Y, Tsumura H, Yamauchi J, Cotecchia S, Koike K, Tsujimoto G, Tanoue A. Alpha1-adrenoceptors are required for normal male sexual function. *Br J Pharmacol* 2007; 152:332-40.
- [39] Legler DF, Bruckner M, Uetz-von AE, Krause P. Prostaglandin E_2 at new glance: novel insights in functional diversity offer therapeutic chances. *Int J Biochem Cell Biol* 2010; 42:198-201.

FIGURE LEGENDS

Figure 1. A) Representative tracings showing the inhibitory effect of prostaglandin E_1 on electrical field stimulation (EFS)-evoked contractions of human vas deferens rings in the absence and in the presence of K^+ channel blockers. B and C summarize the results from all the experiments. Results were calculated as the percentage of the contractile response elicited by EFS (0.25 ms pulse duration, 20 Hz, at a supramaximal voltage of 20 Vcm^{-1} , train duration 5 s) before the addition of prostaglandin E_1 . Values are presented as the mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group.

Figure 2. Graphical representation of the inhibition of EFS-induced contractions by increasing concentrations of prostaglandin E_2 in the absence (control) and in the presence of K^+ channel blockers. Results were calculated as the percentage of the contractile response elicited by EFS before the addition of prostaglandin E_2 . Values are presented as the mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group.

Figure 3. A) Representative tracing and B) bar graph showing the inhibitory effects of increasing extracellular K^+ concentration ($[K^+]_o$) from 5.8 to 25.8 mM on electrical field stimulation (EFS)-induced contractions. After wash out (W) with fresh Krebs-Henseleit solution ($[K^+]_o = 5.8$ mM), the mean amplitude of contractions induced by EFS was reverted to initial conditions. C) Representative tracing and D) bar graph showing the contractile responses to EFS in the absence (control) and in the presence of ouabain ($10^{-7} - 10^{-6}$ M). Results in B) and D) were calculated as the percentage of the contractile

response elicited by EFS before the addition of K^+ or ouabain. Values are mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group. $*P<0.05$ versus control.

Figure 4. Inhibition of electrical field stimulation (EFS)-induced contractions by prostaglandin E_1 (PGE_1), prostaglandin E_2 (PGE_2) and increasing extracellular K^+ concentration ($[K^+]_o$) from 10.8 to 25.8 mM in the absence (control) and in the presence of ouabain (10^{-6} M). Results were calculated as the percentage of the contractile response elicited by EFS before the addition of PGE_1 , PGE_2 or K^+ . Values are mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group.

Figure 5. A) Recordings illustrating the contractile effects of norepinephrine in the absence (control, extracellular K^+ concentration $[K^+]_o = 5.8$ mM) and in the presence of $[K^+]_o$ of 25.8 mM or ouabain (10^{-6} M). Concentration-response curves for norepinephrine in the absence (control, $[K^+]_o = 5.8$ mM) and in the presence of B) $[K^+]_o$ of 25.8 mM, and C) in the presence of ouabain (10^{-6} M). Values are mean \pm S.E.M. Numbers in parentheses are the number of subjects in each group. $*P<0.05$ versus control.

Table 1. pD_2 values and maximal contractions elicited by norepinephrine alone (control) and in the presence of prostaglandin E_1 (PGE_1), PGE_2 , ouabain or increased extracellular K^+ concentration ($[K^+]_o$).

| Norepinephrine | $pD_2 \pm SEM$ | Maximum contraction (mN \pm SEM) |
|------------------------------------|-----------------|---------------------------------------|
| Control ($n = 7$) | 5.01 ± 0.10 | 49.7 ± 7.9 |
| PGE_1 (10^{-6} M) ($n = 5$) | 5.11 ± 0.09 | 48.6 ± 5.3 |
| PGE_2 (10^{-6} M) ($n = 5$) | 5.02 ± 0.09 | 47.5 ± 7.4 |
| Ouabain (10^{-6} M) ($n = 5$) | 4.95 ± 0.14 | 49.5 ± 8.3 |
| $[K^+]_o = 25.8$ mM ($n = 5$) | 5.07 ± 0.10 | 47.9 ± 9.1 |

Values are means \pm SEM. n , number of subjects.

Figure











